Evaluating EDTA Treatment of Sera in FLOW Crossmatches

Maureen M. Miller, John Raposa, Gloria Frankson, Kathleen Gerolami, Kim McGowan, Magdalena Sciupider, Maria Warner, Niketa Sareen, Laurine M. Bow, PhD; Yale University School of Medicine, New Haven, CT

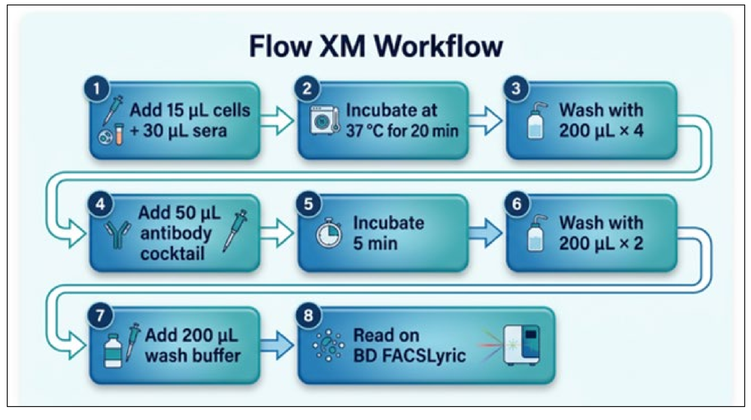
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Aim

We have observed an uptick in positive Flow T cell XMs on patients with no identified antibody. Our standard follow-up is to perform the testing with non-pronased cells and address B cell reactivity. EDTA has been widely adopted for antibody screening to reduce complement interference; we evaluated its use to reduce nonspecific reactivity in flow crossmatches (FCXM).

Method

FCXMs were performed on 98 cell/sera combinations by the Halifaster method with pronase (2.35 Kuntz/mL) treated cells. Data was acquired with the BD FACSLyric™ and analyzed with BD FACSuite software. Standard FCXM results were compared to XMs performed with universal treatment of all controls and sera with 5% EDTA. Positive cutoffs were calculated for the EDTA XM based on 3 standard deviations from the mean of the negative control from 37 donors: 132/388 T/B. The cutoffs for the standard FCXM are 122/349 T/B. EDTA treatment increases the negative control readings and markedly reduces the pooled positive control.



For inquiries, contact Maureen.Miller@yale.edu

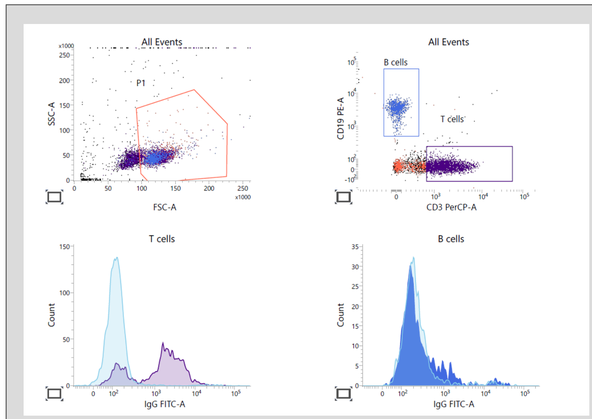
Results

Crossmatch (XM) Results Summary

T XM Summary			B XM Summary		
Total T XM		98	Total B XM		98
Initial Neat-Positive Results and EDTA Follow-up			Initial Neat-Positive Results and EDTA Follow-up		
Positive — neat	35 out of 98	35	Positive — neat	31 out of 98	31
Positive — EDTA		22	Positive — EDTA		26
Negative — EDTA (among initial positives)		13	Negative — EDTA (among initial positives)		5
Initial Neat-Negative Results and EDTA Follow-up			Initial Neat-Negative Results and EDTA Follow-up		
Negative — neat	63 out of 98	63	Negative — neat	67 out of 98	67
Negative — EDTA		63	Negative — EDTA		65
Positive — EDTA (among initial negatives)		0	Positive — EDTA (among initial negatives)		2

Concordance of the FCXM results between EDTA treated sera vs non-treated sera was 87% for TXMs and 93% for BXMs.
Thirteen TXMs were reduced from positive to negative with treatment. Six samples had no antibodies, and EDTA treatment clarified the results, especially for patients with autoimmune diseases such as lupus and IgA nephropathy. These patients were all living recipients with negative virtual crossmatches. Seven patients with possible low-level DSA had channel shifts below the positive threshold after EDTA treatment. Weak antibodies, particularly in re-transplant patients, need further investigation into clinical significance.
There was no instance of a negative TXM converting to positive with EDTA.
Five BXMs were reduced from positive to negative. One had no antibodies to Class II. One had a weak antibody to DR4 and 383 channel shifts, just below the cutoff of 388. Three patients had weak Class I antibodies and BXM channel shifts >300.
Two BXMs changed from negative to positive with EDTA treatment. One sample had a weak Bw4 antibody and would have been T pos / B neg without EDTA and after treatment both XMs are positive with increased TXM channel shifts. The second patient displayed auto reactivity.

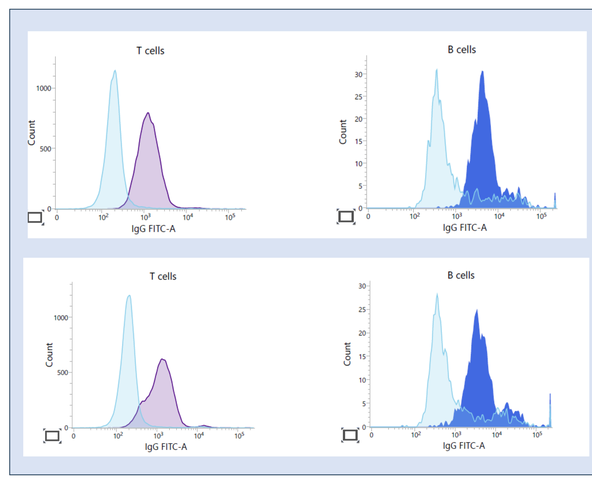
FLOW XM Results



PT Hx: cPRA 0% Lupus

Neat Serum

EDTA Treated Serum



POS XM DSA Bw4

Neat Serum

EDTA Treated Serum

Conclusion

EDTA treatment is an efficient method to reduce non-specific interference in the FCXM and may clarify T positive/B negative flow crossmatch interpretation. More testing is needed to investigate weak antibodies and clinical significance.