

# CRUDE SPLENIC LYMPHOCYTES VERSUS SPLENIC B-CELLS FOR RENAL CROSSMATCHING

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## Introduction

For several decades we have successfully used unseparated splenic lymphocytes (USL) for the complement dependent cytotoxic (CDC) crossmatch (XM) before renal transplantation. However, B-cells separated from spleen using immunomagnetic beads may have increased sensitivity in the CDC assay using shorter test incubation times (Vartdal *et al.*, 1986). This potentially reduces the overall time taken for cadaver renal donor testing. However, prior to changing our well-established XM procedures we compared the two techniques.

## Method

USLs and B-cell XMs were done in parallel on 151 donor spleens and 183 patients using 844 different sera. B-cells were separated from donor spleens using HLA Class II Dynabeads (Dyna). Donors were specifically selected for their patients so the number of positive Xmes was low. Therefore, 6 spleen samples were tested against an additional 28 sera from selected patients with HLA antibodies (PRA 23-78%). The total number of valid XMes was 1,006.

Our NIH CDC method used untreated and DTT treated sera, pre- and post-complement incubations of 30+120 min for USL, 30+60 min for B-cells, and 'Workshop' scoring. Numbers of positive and negative results were compared. Reaction strength scores of 2 and 4 combined were compared with scores of 6 and 8 combined.

## Results

Without DTT treatment 75% of sera were negative and 20% positive in both USL and B-cell Xmes (Table 1); 8 sera were positive with B-cells but negative with USLs; 44 were positive with USLs but negative with B-cells (r-value = 0.86).

With DTT treatment 86% of sera were negative and 13% were positive with both USL and B-cells; 4 sera were positive with USL but negative with B-cells and 5 were positive with B-cells but negative with USLs (r-value = 0.96).

Of the post-DTT discrepancies, 4/5 USL negative, B-cell positive, XMes were strongly positive whereas only 1/4 USL positive, B-cell negative XMes was strongly positive.

Without DTT treatment, 61% of positives were of equivalent strength in both B-cell and USL XMes; 57 XMes were stronger with USL and 41 were stronger with B-cells (Table 2).

For DTT treated sera 64% of positives were of equivalent strength, 10 XMes were stronger with USLs and 36 were stronger with B-cells.

**Table 1. Comparison of positive and negative crossmatches using unseparated splenic lymphocytes (USL) and B-cells and DTT treated and untreated sera.**

USL	B-cell	Untreated		DTT treated	
		No.	%	No.	%
-	-	754	75	867	86
+	+	200	20	130	13
-	+	8	1	5	<1
+	-	44	4	4	<1

**Table 2. Comparison of crossmatch 'strength' using unseparated splenic lymphocytes (USL) and B-cells and DTT treated and untreated sera.**

XM strength	Untreated		DTT treated	
	No.	%	No.	%
USL > B-cell	57	23	10	7
B-cell > USL	41	16	36	26
USL = B-cell	154	61	88	64

## Comments

Overall, Dynabeaded B-cells have equivalent or greater sensitivity to unseparated splenic lymphocytes. In addition, they require shorter incubation times and are 'easier' to read. This all reduces turn around times for cadaver renal donor testing by at least one hour. On this basis crossmatching in this laboratory is now performed using splenic B-cells.

## Reference

Vartdal, F. *et al.* (1986) HLA class I and II typing using cells positively selected from blood by immunomagnetic isolation-a fast and reliable technique. *Tissue Antigens*, **28**, 301.