Comparative analysis of serum pretreatment strategies to remove non-specific serum interference in HLA antibody assessment

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1. INTRODUCTION

Assessment of HLA antibodies occasionally encounter interference from high serum background levels, and non-specific binding, hindering precise HLA-specific reactivity evaluation in solid-phase bead assays. To mitigate this, various strategies are employed.[1,2] We aim to compare the efficacy of Adsorb OUT (AO) and PreSorb™ (PS) (One Lambda™), serum pre-treatment reagents, in mitigating non-specific serum interferences in the HLA single antigen bead (SAB) assay.

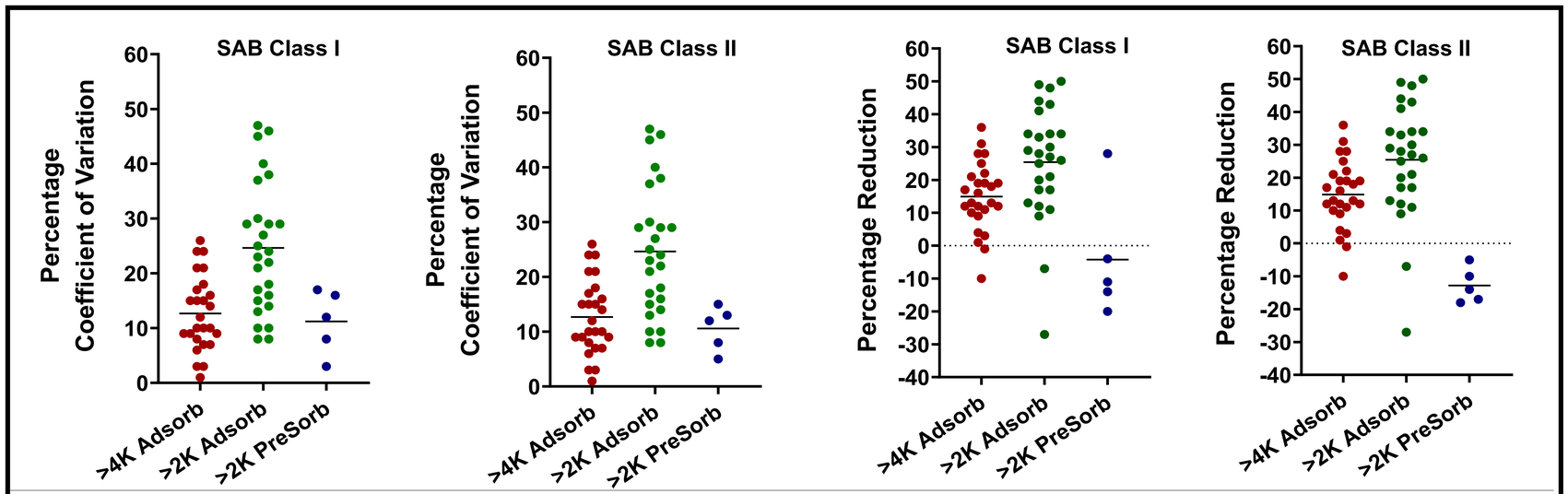
2. METHOD

Twenty-seven deidentified sera samples (22 HLA Class I, 23 HLA Class II) from 25 waitlisted transplant recipients were analyzed using a Single Antigen Bead (SAB) assay (One Lambda™). The set included 17 sera with high background, questionable reactivity, or unexpected crossmatch results, 3 with pan HLA DR reactivity, 5 with typical HLA reactivity (cPRA >50%), and 2 control sera. Sera were tested untreated and post AO and PS pre-treatment. percentage change, and coefficient of variation were calculated. An improvement in serum antibody reactivity was defined as a reduction of non-specific background (NC bead MFI and/or PC to NC ratio) to levels acceptable by the laboratory's SOP (NC<I,500 MFI and/or PC/NC ratio 10), elimination of auto-specificities, reduction in the number of questionable specificities, or a clearer determination of specificity following serum treatment. Additionally, to assess the impact of AO and PS on true HLA-specific antibody reactivity, sera demonstrating normal HLA-specific reactivity were analyzed using the SAB Class I and Class II assays, respectively.

3. RESULTS

Pre-treatment with PS did not significantly affect the strength of the clinically relevant (MFI>2000) HLA antibodies. Pre-treatment with AO tends to reduce MFI. However, for HLA specificities with MFI >4000, the reduction remained within assay variability (%CV ≤20; %Change ≤20), with slightly higher impact on weaker antibodies (MFI 2000-4000; %CV ≤25; %Change ≤25%). **Figure-1.** The comparative analysis showed that only 14% of sera improved with both Adsorb Out™ (AO) and PreSorb™ (PS), while roughly half of the sera showed no improvement with either. **Table 1**. Analysis of serum samples exhibiting non-specific pan-HLA-DR reactivity showed that pan-DR reactivity was removed in all 3 samples (3/3) after PreSorb™ treatment. **Table 2**.

Figure-1 Differences in MFI of specific HLA Class I & II reactivities between untreated and AO/PS treated sera.



*Data for AdsorbOUT™ were taken from samples run in different experiment.

Table-1 Sera with high background exhibited improvement following treatment with AO and/or PS treatment for both HLA Class I and Class antibody assays. FCXM, Flow Cytometric Crossmatch; NC, Negative Control.

Table 1 (a) Class I						
Reason for Testing	**Improved with AO**	**Improved with PS**	**Improved with AO & PS**	**Not Improved with AO & PS**	**Improved with AO or PS**	**Total Samples**
NC Background	3	4	2	6	5	11
Questionable reactivity	0	0	0	2	0	2
Unexpected FCXM results	0	1	0	0	1	1
Total (%)	**3 (21)**	**5 (36)**	**2 (14)**	**8 (57)**	**6 (43)**	**14 (100)**
Table 1 (b) Class II						
NC Background	4	3	2	5	5	10
Questionable reactivity	0	1	0	0	1	1
Unexpected FCXM results	0	1	0	0	1	1
Total (%)	**4 (29)**	**5 (36)**	**2 (14)**	**5 (36)**	**7 (50)**	**12 (100**

Table-2 Differences in MFI of HLA DR and Non-HLA DR reactivities between untreated and PS treated sera.

	HLA DR %CV	**NON-HLA DR % CV**	**HLA DR %Chage**	**NON-HLA DR %Change**
Sample-1	98	14	81	14
Sample-2	124	12	93	16
Sample-3	117	19	90	20
Overall Effect	**113**	**15**	**88**	**17**

*Data is an average of all HLA antigens/beads

CONCLUSIONS:

1. **Serum pretreatment with PreSorb™ did not impact specific HLA Class I and II antibody reactivities.**
2. **Neither Adsorb OUT nor PreSorb™ is superior in universally reducing non-specific reactivities, suggesting their complementary usage.**
3. **PreSorb™ outperformed Adsorb OUT in select cases of non-specific reactivity and proves effective in removing pan DR reactivity.**

REFERENCES: **1.** Zerrouki A, et. al. Transpl Immunol. 2016;36:20-24. **2.** McCaughan J, Hepatobiliary Surg Nutr. 2019;8(1):37-52.