Using PreSorb and MagSort to characterize pan-DR sera

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Introduction

Unexpected binding reactivity might arise from HLA-specific antibodies or non-HLA/autoantibodies/background binding. To resolve this unanticipated pan-DR binding reactivity, we utilized One Lambda's PreSorb, LABScreen Single Antigen (SAB), LABScreen PRA, LABScreen C1qScreen, and MagSort treatment on six different serum cases.

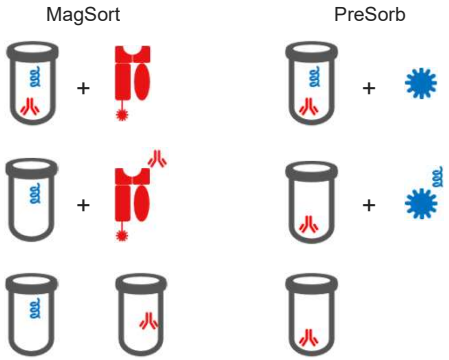
Materials and Methods

PreSorb treatment consists of incubating each serum with PreSorb beads for 10 minutes and recovering serum using a centrifuge and magnetic rack.

MagSort treatment consists of incubating each serum with HLA-specific MagSort beads for 120 minutes. After washing the beads, HLA-specific antibodies were eluted.

Recombinant cell treatment consists of incubating each serum with cells expressing specific HLA for 120 minutes. After washing the cells, HLA-specific antibodies were eluted.

Pre-treated sera samples and treated samples were then tested using LABScreen Single Antigen, LABScreen PRA, and LABScreen C1qScreen.



Results

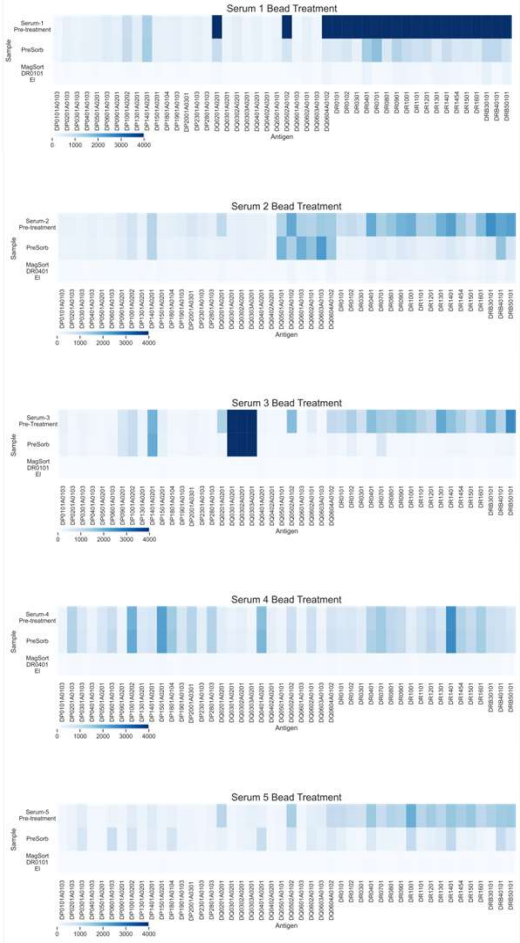


Figure 1: Heat map showing pre-treatment MFI levels, levels following PreSorb treatment, and MagSort elution levels

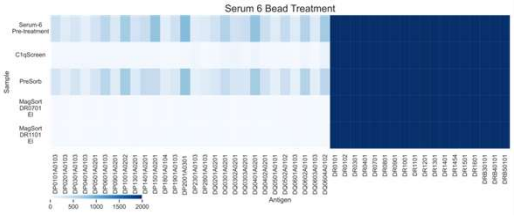


Figure 2: Pan-DR reactivity consistent across all assays and serum treatment options.

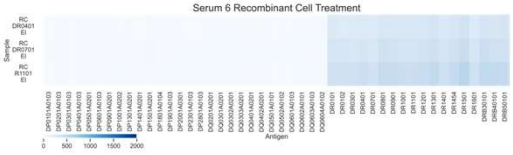


Figure 3: Antibody absorption elution carried out using recombinant cells on serum 6.

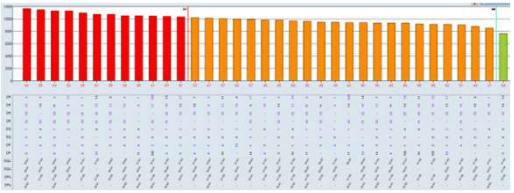


Figure 4: Testing with LS1PRA also demonstrated reactivity across all specificities for serum 6.

PreSorb treatment effectively decreased the unexpected pan-DR binding reactivity in five sera (Figure 1). In those five sera, MagSort beads targeting DRB1*01:01 and DRB1*04:01 did not isolate DRB1-specific antibodies, confirming that these are not HLA specific signals.

However, PreSorb treatment did not decrease the pan-DR binding reactivity in serum 6 (Figure 2). C1qScreen assay indicated that these pan-DR antibodies interact with the complement factors. MagSort beads targeting DRB1*07:01, DRB1*11:01, and DRB1*15:01 could also isolate the pan-DR antibodies. Recombinant cells expressing DRB1*04:01, DRB1*07:01, and DRB1*11:01 isolated the antibodies albeit with low efficiency based on SAB mean fluorescence intensity (MFI) values (Figure 3). LABScreen PRA with antigens from different cell lines confirmed the sample pan-DR specificity (Figure 4).

Discussion

PreSorb and MagSort can effectively resolve pan-DR binding reactivity for the five serum cases. In the case of serum 6, all treatments indicated the presence of genuine pan-DR antibodies which is puzzling given the patient's typing of DRB1*07:01 and DRB1*11:01.The difference in levels of SAB signals for recombinant cell vs. MagSort isolation (MFI ~300 vs ~7000) may be due to the higher density of the HLA molecules on the magnetic beads. Alternatively, proteins and lipids associated with cellular HLA could hinder the interaction of pan-DR antibodies. While we cannot dismiss the possibility that the conformation of HLA molecules on the beads differs from that on the cells, the weak binding of pan-DR antibodies to the cells may be significant for researchers.

Conclusion

In summary, these tools help our understanding and validation of both HLA specific and non-specific binding patterns.

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