



Back to the Basics? Resolving a novel non-specific reactive HLA antibody pattern using Fetal Bovine Serum

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INTRODUCTION

Historically, non-specific HLA antibody reactivity has been observed in Single Antigen Bead (SAB) assays with serum pre-treatments, including fetal bovine serum (FBS) and PreSorb, offering some resolution. Recently, we have observed a recurrent pattern within LabScreen HLA class II SAB assays unique to Lot 17, with positivity towards DRB1*01:02, DRB1*04:06, DRB3*02:02, DRB1*10:01, DRB1*12:01, and DRB1*01:01 in patient serum samples (Figure 1). This reactivity was seen in patients with negative Flow PRA and included instances where one or more of the above specificities were auto-reactive.

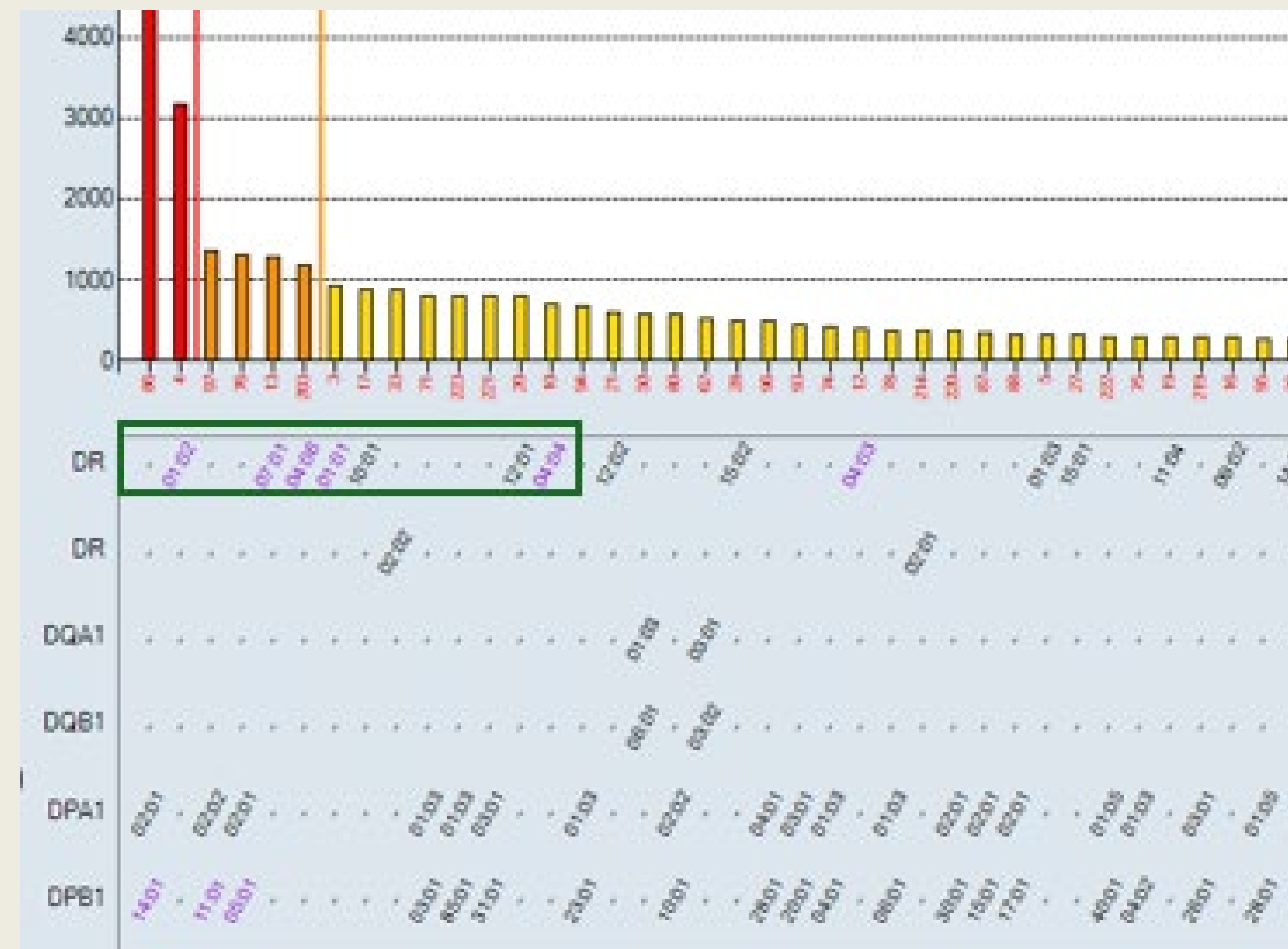


Figure 1 Single Antigen Bead class II showing the recurrent non-specific pattern

METHODS AND MATERIAL

Patient serum samples were tested for anti-HLA antibodies by LabScreen Class II SAB (One Lambda). All sera were treated with EDTA.

Fetal Bovine Serum (Corning Lot# 09524001) was used for serum treatment. 40µL of patient's serum was incubated with 2µL of FBS for 30 minutes prior to incubation with 10µL of beads (Labscreen SAB II + Explex).

RESULTS

Initial investigations indicated that this non-specific pattern was unique to Lot 17 of LabScreen Class II SAB, which was confirmed by repeat testing using Lot 17 reagents for a sample that lacked the non-specific pattern when tested with Lot 16 (Figure 2). Testing with Lot 17 SAB now showed the above non-specific pattern. PreSorb treatment was ineffective in clearing the non-specific reactivity (Figure 3).

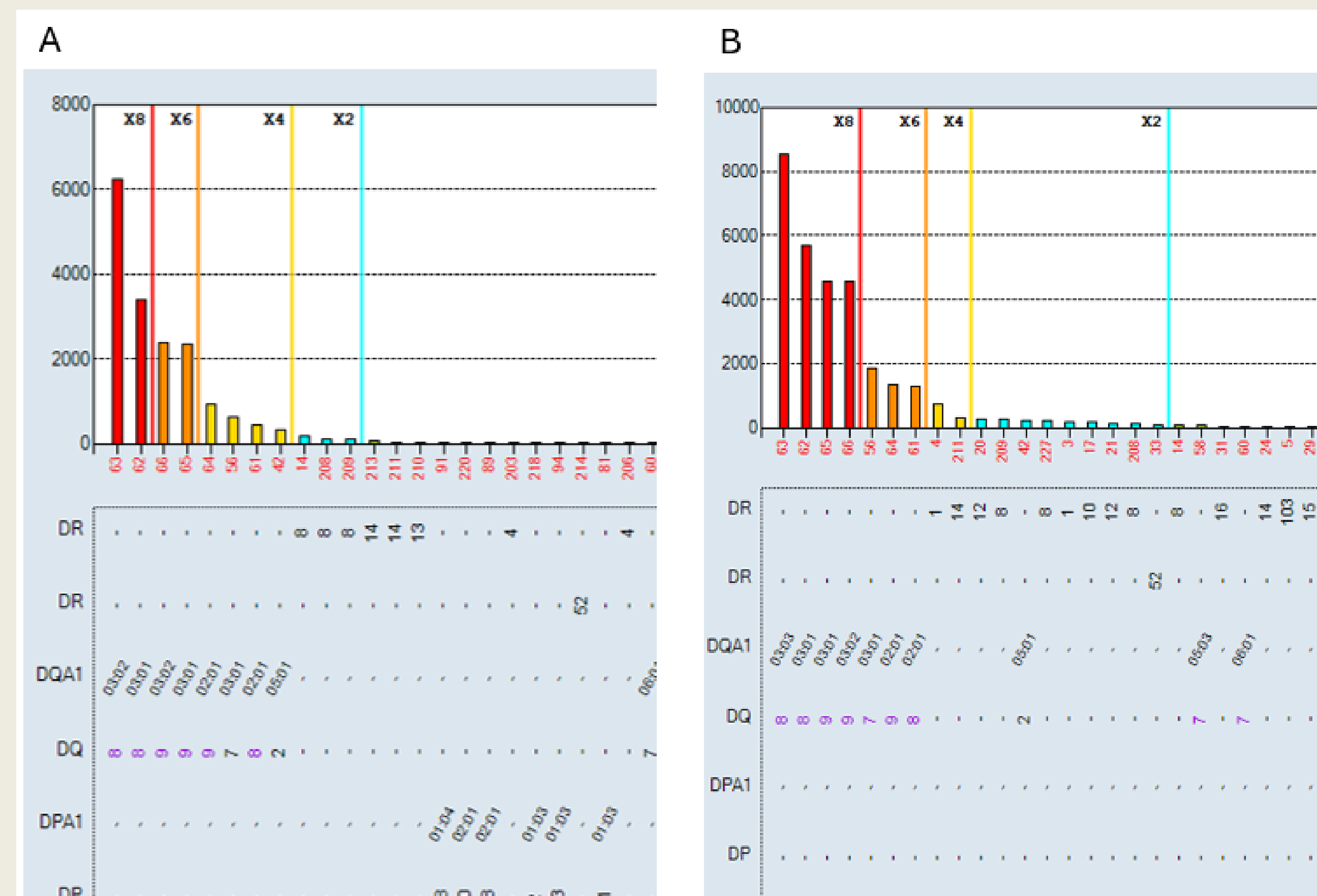


Figure 2 Sample showing the non-specific pattern tested (A) with Lot 16 and (B) with Lot 17 LabScreen Class II SAB

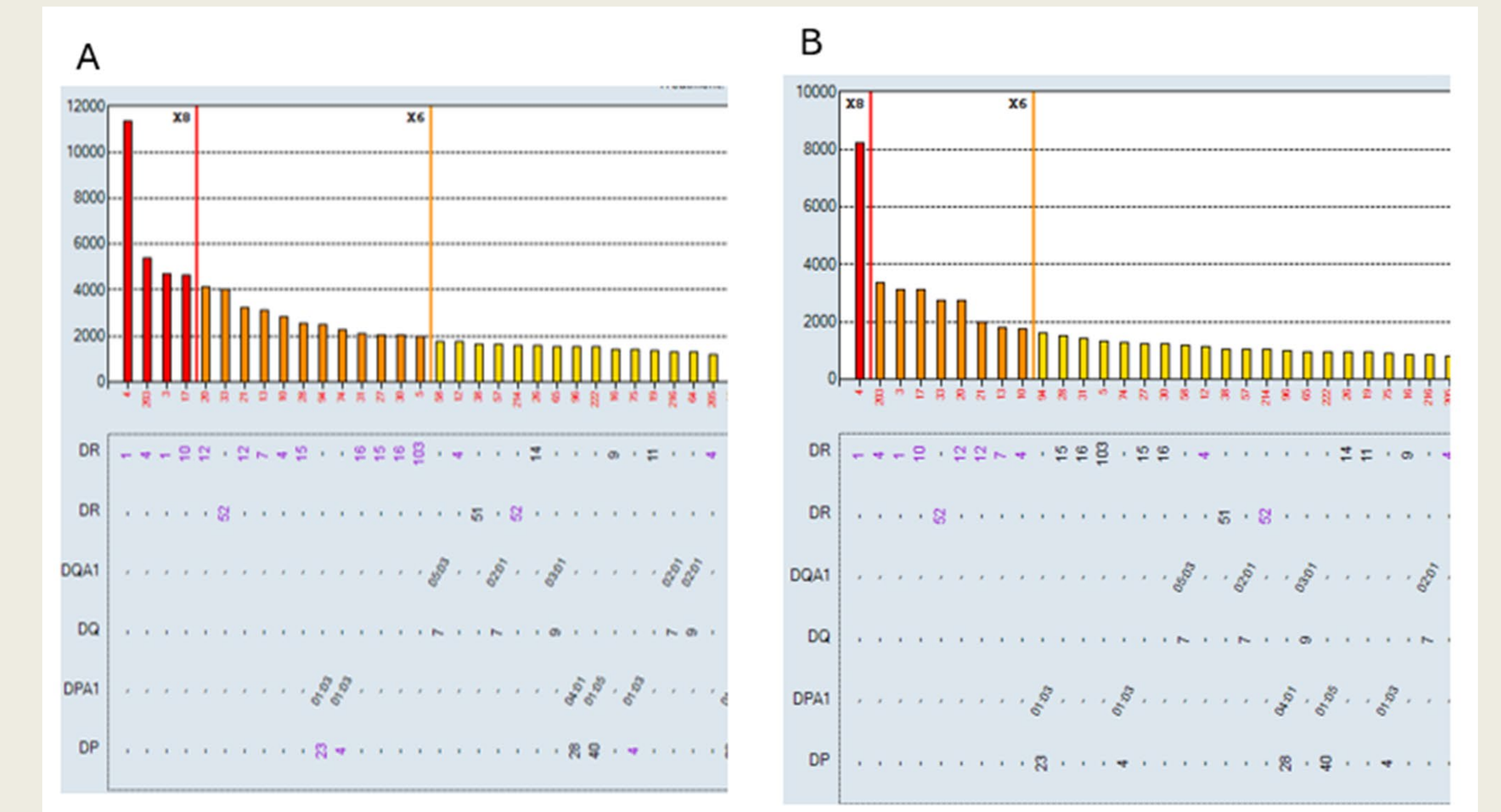


Figure 3 Sample showing the non-specific pattern tested (A) without and (B) with PRESORB treatment

Herein, we further describe efforts to resolve this non-specific pattern in a lung transplant recipient being monitored for donor specific anti-HLA antibodies. Donor HLA class II antigens were DR17, DR15 DRw52, DRw51 DQ2, DQ6. Class II DSA interpretation was difficult due to the possible DSA comingling within apparent non-specific reactivity (Figure 4). PreSorb treatment was ineffective in clearing the non-specific reactivity. Previous reports have indicated that pretreatment of serum with Fetal Bovine Serum (FBS) reduces non-specific background and enhances HLA antibody detection. Therefore, the patient's serum was pre-incubated with Fetal Bovine Serum (FBS) prior to single antigen bead assay testing. FBS treatment effectively reduced the non-specificity and allowed a clear detection of DQ2 DSA.

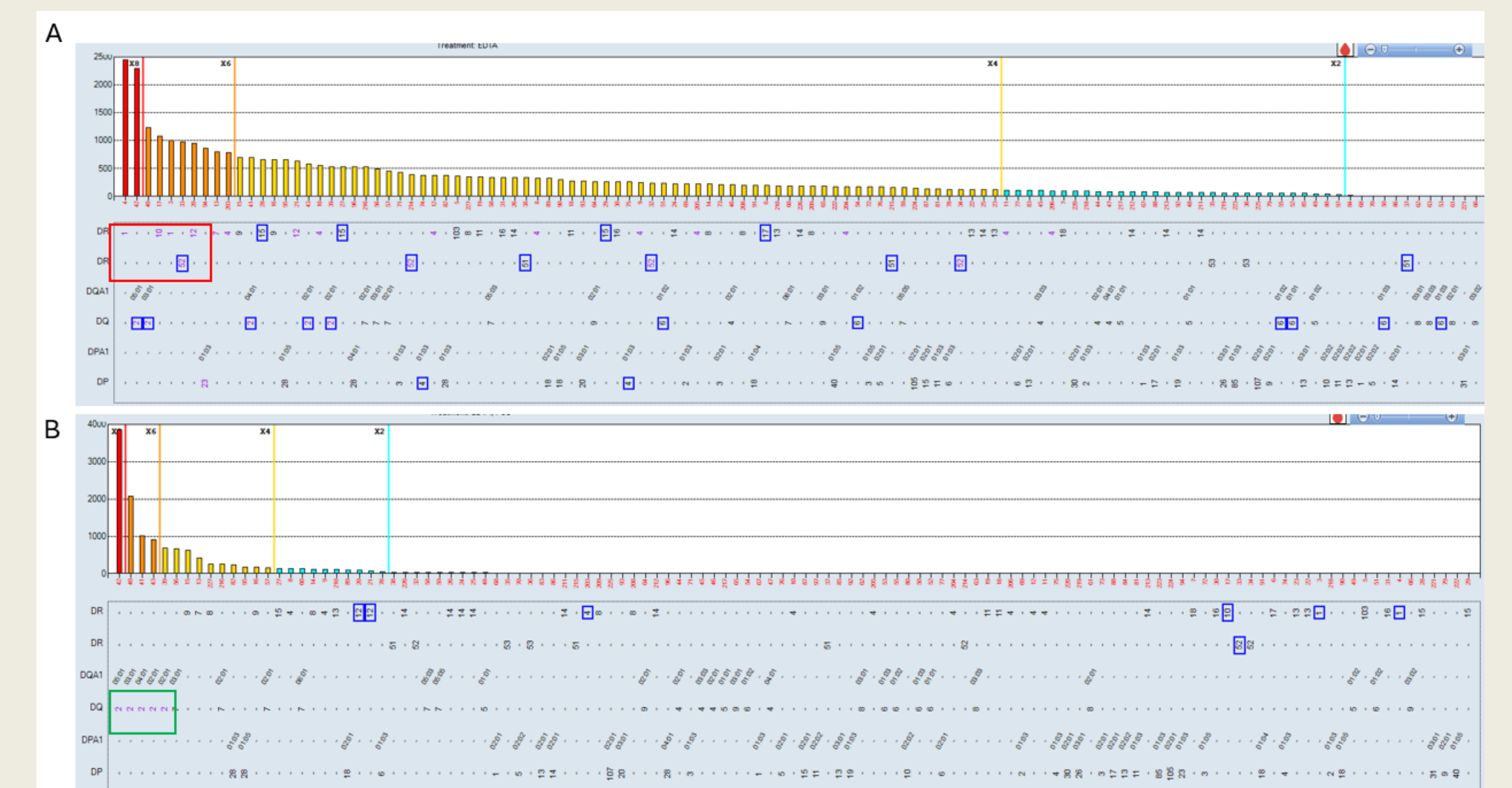


Figure 4 Patient's HLA class II antibodies (A) without and (B) with FCS treatment. A- non-specific pattern indicated in red rectangle, and DSA in blue squares. B-non-specific pattern antigens in blue squares, and DSA in green rectangle.

We further confirmed the benefit of FBS serum pre-treatment to resolve the non-specific pattern in an additional 10 samples. Finally, to simplify the testing workflow, we confirmed that FBS pre-treatment of the single antigen beads, rather than the serum, similarly eliminated this non-specific pattern.

CONCLUSIONS

This case highlights the necessary vigilance needed to assess lot-to-lot variation, including awareness of possible new non-specific patterns and the importance of robust parallel testing with new lots. It also underscores the value of understanding the limitations of the solid phase antibody testing for accurate interpretation of HLA antibodies.

REFERENCES

Jaramillo A., House J., Nelson L., Stoll S., Ridgley R., Khamash H. Serum Treatment with Fetal Bovine Serum Significantly Increases Luminex-Based HLA Antibody Assay Specificity by Reducing Heterophile Antibody Interference Am J Transplant. 2017;17 (suppl 3).