Is it time to go light on the peanut butter (effect)

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Introduction

The single antigen bead (SAB) assay is the gold standard for identifying HLA specific antibodies. A proposed limitation of the assay is the potential reduction in apparent antibody mean fluorescence intensity (MFI) due to dilution across SABs expressing shared HLA epitopes (aka the "peanut butter effect"). If present, this proposed phenomenon could underestimate antibody strength and affect concordance between virtual crossmatch (VXM) and cell-based crossmatch (CBXM) results. However, this hypothesis remains unproven.

Study Aim: To evaluate the relevance of the "peanut butter effect" by determining whether antibody dilution across SABs influences MFI values.

Materials and Methods

Six sensitized patients with well-characterized sera were selected (3 for class I and 3 for class II HLA antibody analysis). All sera were pre-treated with EDTA to prevent complement mediated inhibition. Parallel testing of sera (20µl/reaction) was performed using the Rapid Optimized SAB (ROB) assay with either 5µl (standard) or 1µl (5-fold reduced antigen) of beads, allowing assessment of the impact of a 5-fold difference in HLA antigen levels on antibody MFI.

Results

Trimmed MFI data from all 6 sera were stratified by MFI and compared between the 1µl vs 5µl SAB conditions. Overall, only a modest increase in MFI was observed with 5-fold lower antigen levels (1µl vs 5µl) for both HLA class I and class II antibodies (Figure 1).

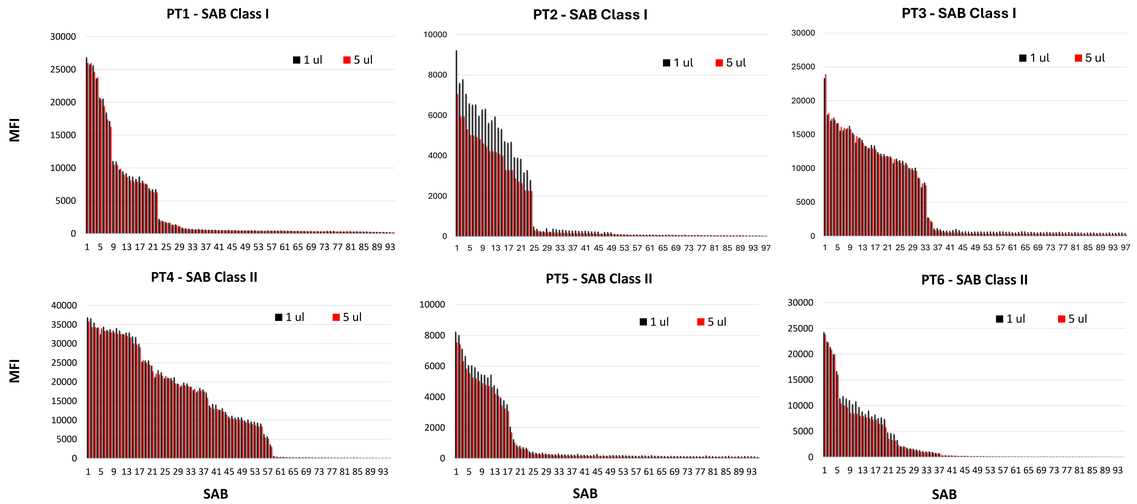


Figure 1: Single antigen bead assay MFI data comparing 5-fold reduced (1µl; black bars) and standard (5 µl; red bars) SAB conditions for patients with HLA class I and HLA class II antibodies.

The MFI correlation between 1µl vs 5µl bead conditions was excellent for all six sera with R^2 values of > 0.99 (Figure 2).

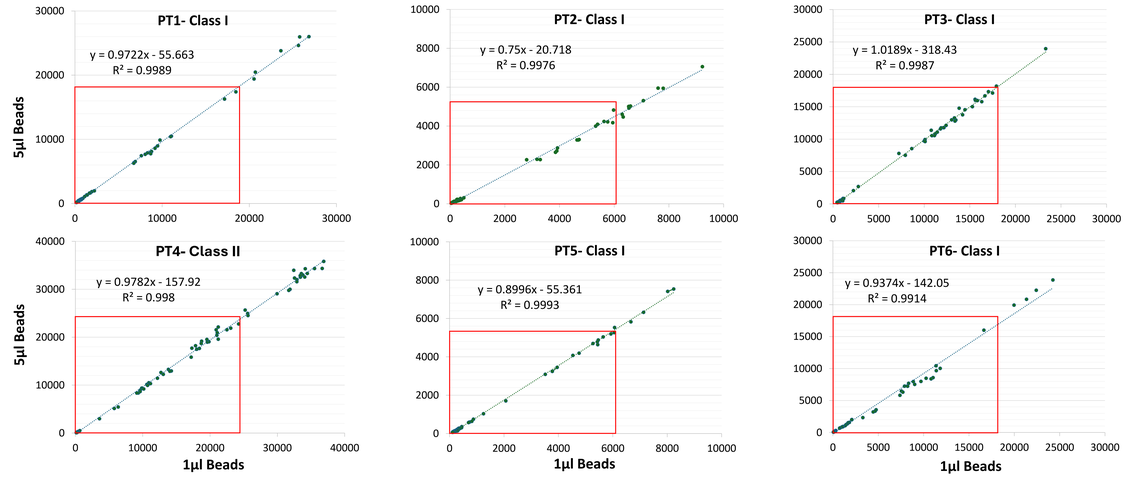


Figure 2: Comparison plots between 1µl and 5µl bead conditions tested using the ROB SAB assay demonstrate excellent MFI correlation. A slight bias towards higher MFI for the 1 µl condition is seen for some samples.

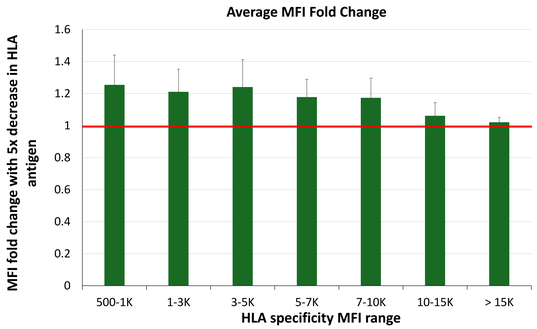


Figure 3: Average MFI fold increase with 5-fold lower antigen, stratified by SAB MFI.

When stratified by antibody strength, the greatest effect was observed for low-level antibodies (500 – 5,000 MFI), with only a modest 21–25% increase in average MFI seen under the lower antigen (1 µl) condition.

The increase was even smaller for intermediate-level antibodies (17%) and negligible for strong antibodies (>10,000 MFI) (Figure 3).

The study findings indicate that HLA antibody dilution across SABs has minimal impact on MFI values.

Conclusion

This study demonstrates that a 5-fold reduction in HLA antigen levels in the SAB assay results in only modest increases in antibody MFI, even for weaker HLA antibodies. These findings indicate that antibody dilution across beads sharing common eplets has minimal impact on MFI values and suggest that the "peanut butter effect" is unlikely to have a meaningful impact on SAB assay results or be a major cause of discordance between VXM and CBXM results. Instead, discrepancies are more likely attributable to inherent differences between the assay platforms.