Use of Titration for Improving Quantitative Evaluation of Anti-HLA Antibodies by SAB

Janara Mehrabli[1], Joseph Abraha,[1] Clarkson Crane[2] , Gerald Morris,[1]
University of California at San Diego, San Diego, CA

Introduction

Multiplexed flow cytometry-based single antigen bead (SAB) immunoassays are the current standard for detection and identification of anti-HLA alloantibodies. While highly sensitive and specific, well-recognized limitations of SAB including non-linearity, saturation, reagent variability, and potential for assay inhibition limit quantitative determination of alloantibody concentrations using the assay output of Mean Fluorescence Intensity (MFI). This limitation is a barrier to accurate assessment of potential clinical impact of anti-HLA antibodies. We hypothesized that anti-HLA antibody quantification by titration, as compared to MFI, will be a more informative measure of antibody quantity.

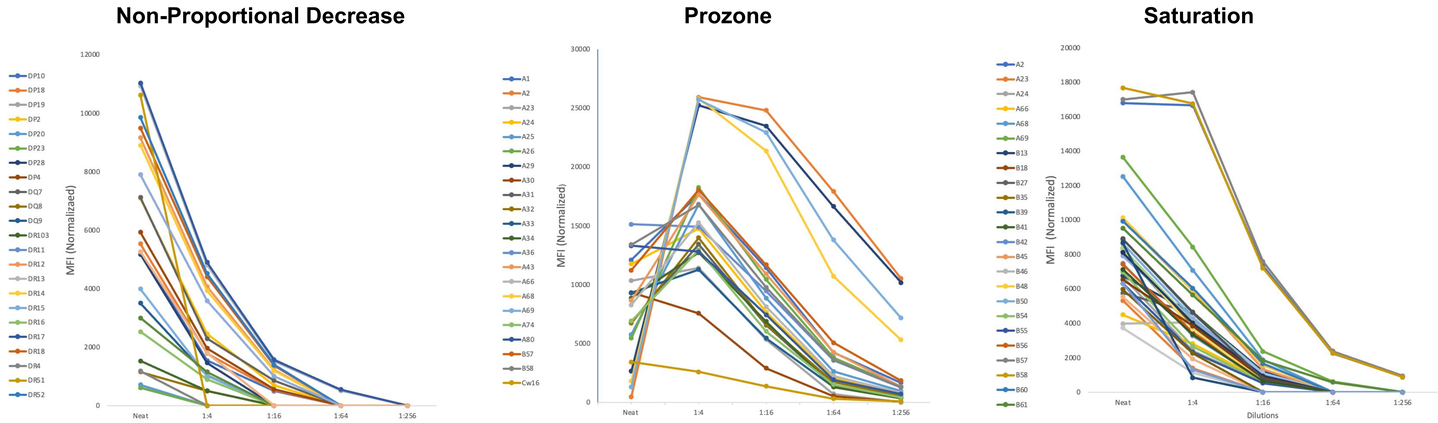
Methods

Study Samples
We examined the utility of SAB titration in 19 serum samples from adult patients listed for kidney transplantation with known high levels of individual anti-HLA antibodies (MFI > 8,000 and/or present at 1:10 serum dilution).

Anti-HLA Antibody Testing
SAB testing was performed using LABScreen Single Antigen Class I and Class II reagents on a FLEXMAP 3D analyzer with each serum sample was tested by a serial 4-fold dilution (neat, 1:4, 1:16, 1:64, 1:256). Antibodies detected at > 1000 MFI at any dilution (n = 625) were included in the analysis.

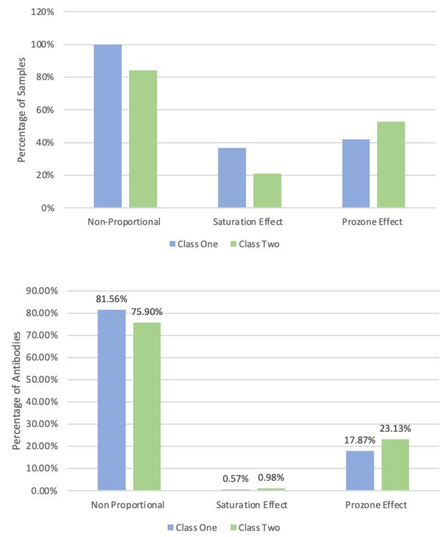
Results

Fig 1: Dilution Tested Identifies 3 Patterns of Antibody Strength



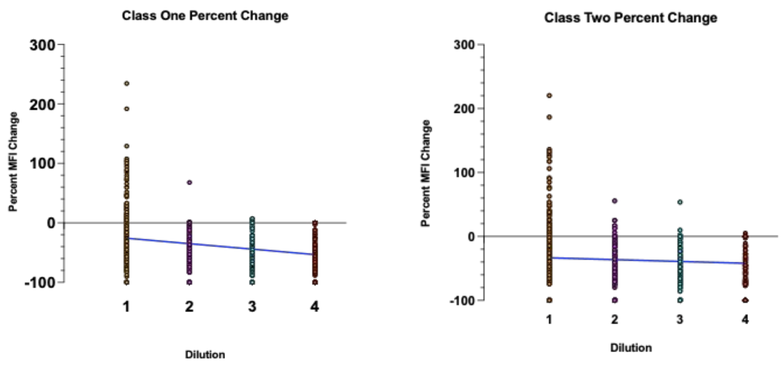
Sample 14 Class II presents an expected dilution pattern as the MFI values decrease as the serum samples are diluted. In this sample, all antibodies are undetectable after a 1:256 dilution. Sample 9 Class I antibodies revealed a Prozone effect in 18 out of 22 detected antibodies as they had falsely reduced neat MFI values when compared to the following 1:4 dilution. Sample 11 Class I had an example of saturation as the MFI value remained around 16000 despite a following dilution.

Fig 2: Incidence of Antibody Dilution Strength Patterns



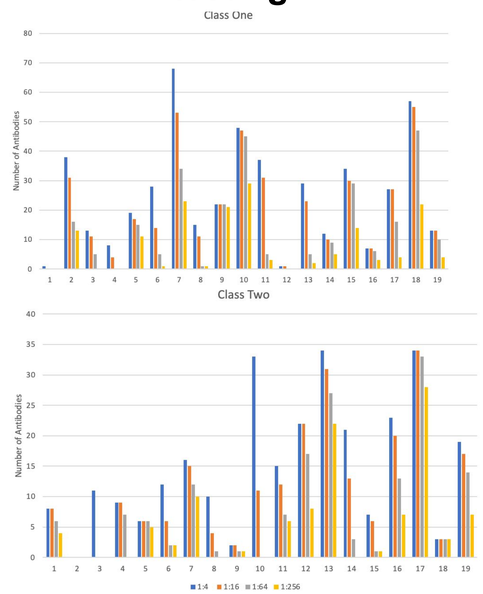
Prozone effect was observed for at least one antibody specificity in 8/19 class one samples (42%) and 10/19 class II samples (53%).Saturation was detected for at least one antibody specificity in 7/19 class one samples (37%) and 4/19 class II samples (21%).

Fig 3. Non-Linear relationship between MFI and dilution



The decrease in percent change presents an unexpected non-linear relationship between dilution and MFI. In a four-fold dilution, the expected decrease in MFI of 75% after the first dilution was often not detected. As an example, sample 9 class one MFI value increased by +5281% after 1:4 dilution compared to the neat. Overall, class one had an average percent change of -39.53% with a range of +5281% to -100%. Class two had an average percent change of -37.63% with a range of +220.3% to -100%.

Fig 4: Sample patterns of dilution testing



26 antibodies became undetectable after 4-fold dilution (initial MFI values 1179 – 10,617). Several antibodies were persistently detectable at 16- (n = 236, 37.8%), 64- (n = 160, 25.6%), and 256-fold dilutions (n = 70, 11.2%)

Conclusion

- Our results illustrate the limitations in reliance in MFI for evaluation of anti-HLA antibody concentrations.
- Serum dilution and titration by SAB is a more robust measure of anti-HLA antibody concentration.
- The results presented highlight the importance of accurately evaluating anti-HLA antibody concentrations as it has significant clinical implications for assessing pre-transplant immunologic risk, evaluating potential responsiveness to desensitization, and assessing responses to treatment.
- Current work aims to correlate dilution and titration of donor specific antibodies with biopsy proven acute rejection.