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Importance of Defining Center-Specific MFI Ranges in Single-Antigen Bead HLA Antibody Assays

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Abstract

Luminex-based, single antigen bead (SAB) testing for HLA antibodies is performed world-wide. Results are provided as Mean Fluorescence Intensity (MFI) which approximates antibody concentration. Although MFI was not intended as a quantitative measure and is not part of the FDA clearance for such tests, MFI values have been used in clinical decision making. Thus, understanding what constitutes a "meaningful" change in MFI is critical. Since SAB testing is a biological assessment, it is impacted by many extrinsic and intrinsic factors. To understand the impact of such variables, we undertook an exercise to determine the variability of SAB testing at a single center.

Methods and Materials

Three sera containing both class I and II HLA specificities were tested by six technologists as part of an internal QC program. Sera A and B were distinct while C was a repeat of A. All technologists utilized the same SAB lots, same reagents and ran on the same instrument. MFI values for individual beads were collected for all sera and compared across technologists and for the same technologist. In total,1068 Class I and 912 Class II data points were evaluated. Tabulated MFI values ranged from 2000 – 50,000 and the Mean MFI, standard deviation and %C.V. were calculated for each sera and as cumulative values.

Introduction

This study was designed to evaluate the tech-to-tech variability in HLA antibody testing at a single center. Although not approved as a quantitative test, the values obtained from such testing are used in clinical decisions. Using well characterized sera, laboratory technologists' results were compared for consistency/ reproducibility. Our single center study showed that technologists can obtain similar results however, there is a degree of inherent variability. Such differences are, in part, directly related to the actual value measured. That is, variability is highest at the lowest MFI values and lowest at high MFI values. Such information is invaluable to clinicians especially when attempting to determine what "differences" in values represent a statistically significant change.

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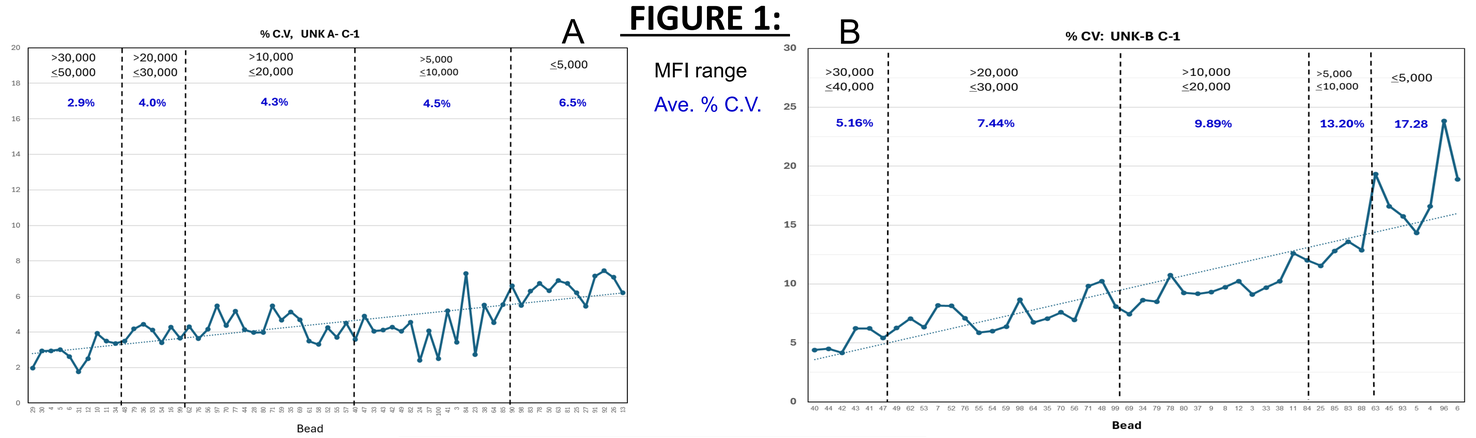
Results

As anticipated, the variability of MFI values, expressed as % C.V., was a function of the MFI and ranged from ~2% to ~17%. The % C.V. was highest for MFI values <5,000 and lowest for MFI values >40,000 (Table 1; representative examples, Figure 1A,B). Overall, there was an excellent correlation between technologists (Fig. 2) and between runs (Fig. 3). Interestingly, the % C.V., across similar MFI ranges, was significantly different between sera **A** and **B** for class I reactivity. The average % C.V. for serum **A** ranged from 3 to 6% (2,500 - 36,000 MFI) while serum **B** ranged from 5 to17% (1,400 – 34,000 MFI). This observation suggests that MFI variability may also be a function of the individual serum tested as well as inherent assay factors.

FIGURE 1:



MFI range
Ave. % C.V.

TABLE 1:

% C.V. range and mean for all sera tested.

	% C.V.			
	Class I		Class II	
MFI Range	Range	Mean	Range	Mean
< 5,000	6.2 - 17.0	11.0	9.6 - 12.0	10.5
>5 - 10,000	4.5 - 13.0	8.0	8.1 - 9.7	5.9
>10 - 20,000	4.3 - 9.9	7.0	5.3 - 8.5	6.3
>20 - 30,000	4.4 - 7.4	5.7	3.2 - 5.4	4.6
>30 - 40,000	3.8 - 5.2	4.3	**	3.0
>40 - 50,000	2.4 - 2.9	2.7	**	1.9

** Only one serum had C-II values >30,000 MFI.

FIGURE 2:

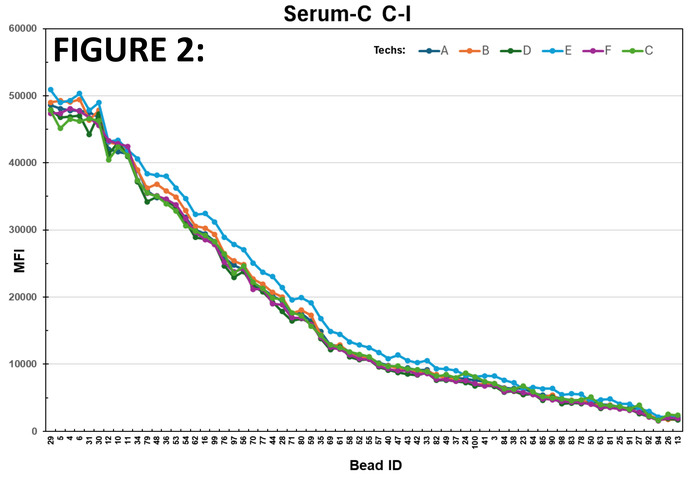
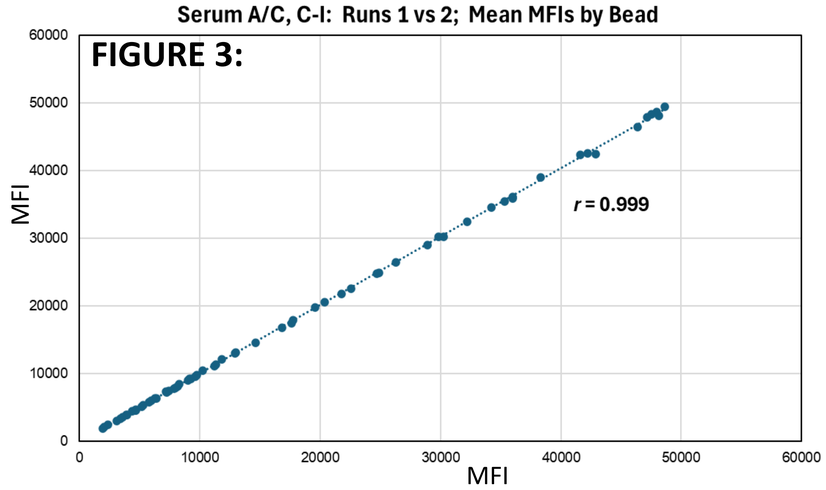


FIGURE 3:



Conclusions

Our data highlights the importance of documenting MFI variability observed in SAB testing at a single center. As changes in MFI values can and are used to guide clinical decisions, understanding what constitutes a "significant" change is critical. For example, at a common cut-off value of 3000 MFI and a 20% C.V., values between 2,400 and 3,600 would be considered as comparable and not considered a meaningful change. In our study we demonstrated that: 1) the % C.V. varies as a function of the MFI value; 2) there was a good correlation of MFI values between technologists at this center; 3) the variability of MFI values may also be a property of the serum/antibody itself. Specifically, at high MFI values, where bead saturation occurs, variability is less while at low MFI values, below bead saturation, reactivity is impacted by antibody affinity/avidity and testing parameters. Finally, understanding center-specific MFI ranges will help clinicians define what constitutes a statistically meaningful change.