

Title:

Validation of the Luminex platform to detect and quantify anti-HLA antibodies:
Consistency in median fluorescence intensity (MFI) among 7 CTOT core laboratories.

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Purpose:

Luminex assays for the detection and quantification of anti-HLA antibodies are widely used to evaluate risks of allograft rejection and to monitor immune responses following transplantation. However, variations in outcome (median fluorescence intensity, MFI) among HLA laboratories are not well defined. The purpose of this study was to determine the correlation of MFI distributions among 7 laboratories (A-G) testing a large number of identical sets of reference sera and reagents.

Methods:

Following adoption of standardized SOPs, HLA class I and II reference sera were exchanged together with identical lots of luminex test reagents (10 kits containing 594 beads from two major manufacturers) to 7 centers across North America. Depending on the nature of the kit (screen v. single antigen and class I v. II), 15 to 22 sera samples were tested per kit, yielding a total of 9,918 MFI values collected from each center. Raw MFI values were analyzed via standard linear regression models, ANOVA and multidimensional scaling (MDS) techniques. (For completeness, mean and standardized FI values were also analyzed producing similar results.)

Results:

The 69,426 MFI values ranged from 0-25459 intensity units (mean±SD = 2844±5050). Systematic differences were observed among the 7 centers: Center B (3139±5429) had a significantly higher average MFI value ($\Delta 295$ MFI, $P < 0.001$); and Centers D (2436±4438) and G (2569±4686) had significantly lower average MFI values ($\Delta -408$ MFI, $P < 0.001$, and $\Delta -275$ MFI, $P < 0.01$, respectively). MDS confirmed these small differences. As shown in Figure, pairwise correlations among centers ranged from 0.953 to 0.992.

Conclusions:

Despite small and inconsequential differences among the centers, the overall consistency in MFI values was excellent ($R^2 > 0.91$), helping to validate the reproducibility of the HLA antibody luminex results.

MFI correlations among 7 centers (A-G)

