Title:
Validation of the luminex platform to detect and quantify HLA antibodies: Effect of standardizing operation procedures on test variability by CTOT core laboratories

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Purpose:
Luminex assays to detect and quantify HLA antibodies are used to evaluate risks of allograft rejection and monitor immune responses following transplantation. The purpose of this study was to assess the relative degree of MFI variation related to standardized (SOP) versus non-standardized 'in-house' operating procedures (HOP) across 5 centers testing identical sera.

Methods:
Luminex single antigen test results from two ASHI proficiency test (PT) surveys were collected from 5 CTOT centers. Each ASHI PT tested 5 sera with HOP and the lots of OneLambda reagents regularly used in each center. Following adoption of SOP, 21 reference sera were exchanged together with identical lots (lot 1 and lot 2) of OneLambda single-antigen test reagents to the 5 centers. From all bead/serum tests, the coefficient of variation (%CV) was calculated using raw MFI values. The %CV was analyzed for HOP- versus SOP-test effects using nonparametric methods.

Results:
Figure 1 shows boxplots of %CV distributions grouped according to OP- and kit-type. In general, MFI variation was more than two times greater in HOP ASHI PT (62% median %CV) than SOP tests (21% median %CV for lot1 and 20% for lot2), P<0.0001. When similar analyses were performed for different MFI ranges (0-500, 501-1000, 1001-3000, 3000-10000, >10000), the highest %CVs were observed in 501-1000 range and lowest %CVs were observed above 10000. Even above 10,000 range, HOP ASHI PT %CV (median 22%) were double those of SOP %CV (lot1 median 7% and lot2 median 9%). In all ranges, %CV in HOP ASHI PT tests were more than two times greater than SOP tests (data not shown). %CV differences between SOP lot1 and lot2 were small.

Conclusions:
Our results suggest that MFI variation can be significantly reduced by adoption of SOP. Further, SOP-lot variation among luminex single antigen beads was small. Implementation of a harmonized approach for assessment of HLA antibodies by luminex will greatly enhance data sharing and analysis across clinical trial sites and for uniformity in diagnostic testing.

%CV Comparison between HOP and SOP
(5 CTOT Core Laboratories)