Title:

Validation of the luminex platform to detect and quantify anti-HLA antibodies: Assessment of Manufacture variability by 7 CTOT core laboratories.

Authors:

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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 florescence intensity, MFI) among manufactures and their products are not well defined. The purpose of this study was to assess the relative degree of MFI variation related to kit type (screening, PRA or single antigen) and vendor (A&B) using results from 7 centers testing identical sets of reference sera and reagents.

Methods:

Following adoption of standardized SOPs, reference sera were exchanged together with identical lots of luminex test reagents to 7 centers across North America. One screening, 2 PRA (Class I and II) and 2 single antigen (Class I and II) kits per vendor (A & B) were analyzed using 15 to 22 sera samples depending on kit type. For each reagent/serum combination (n = 9,918), the coefficient of variation (%CV) was calculated using the 7 center-specific raw MFI values. %CV was analyzed for kit and manufacture effects using nonparametric and quantile regression methods.

Results:

The median %CV across all 9,918 analytes was 25%. The Figure shows boxplots of %CV distributions grouped according to vendor and kit-type varying around the 25% line. In general, MFI variation was greater for Vendor A (median %CV = 30%) than Vendor B (median %CV = 20%), $P<0.001$. For Vendor A, single antigen kits exhibited the highest MFI variation (%CV = 36% and 29% for Class I and II, respectively, $P<0.001$); whereas single antigens kits exhibited consistently low MFI variation (%CV = 20%, $P<0.001$) for Vendor B. Regarding single antigens, A2, A68, Cw12 and DQ4 reagents tended to exhibit more variability than other specificities when analytes were 'positive' (MFI values above 1000, data not shown). When one vendor was used to establish specificity truth for single antigen class I and class II beads the overall correct classification of specificity assignment was >90% and the AUC was ≥ 0.9.

Conclusions:

Our results suggest that MFI variation depends on both the manufacture and the type of kit. When data was re-analyzed following the Vendor's standardization protocols, results were similar to those reported above. The development of robust standardization methods is necessary to directly compare luminex MFI results across commercially available kits.