Title:

Cross validation of Luminex platforms from two vendors to detect and quantify HLA antibodies using single antigen assay results from 7 CTOT core laboratories.

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Aims:

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 degree of agreement in a quantifiable metric (median florescence intensity, MFI) related to single antigen assays across two manufacturers (Vendor A and B) using results from centers testing identical sets of sera and reagents.

Methods:

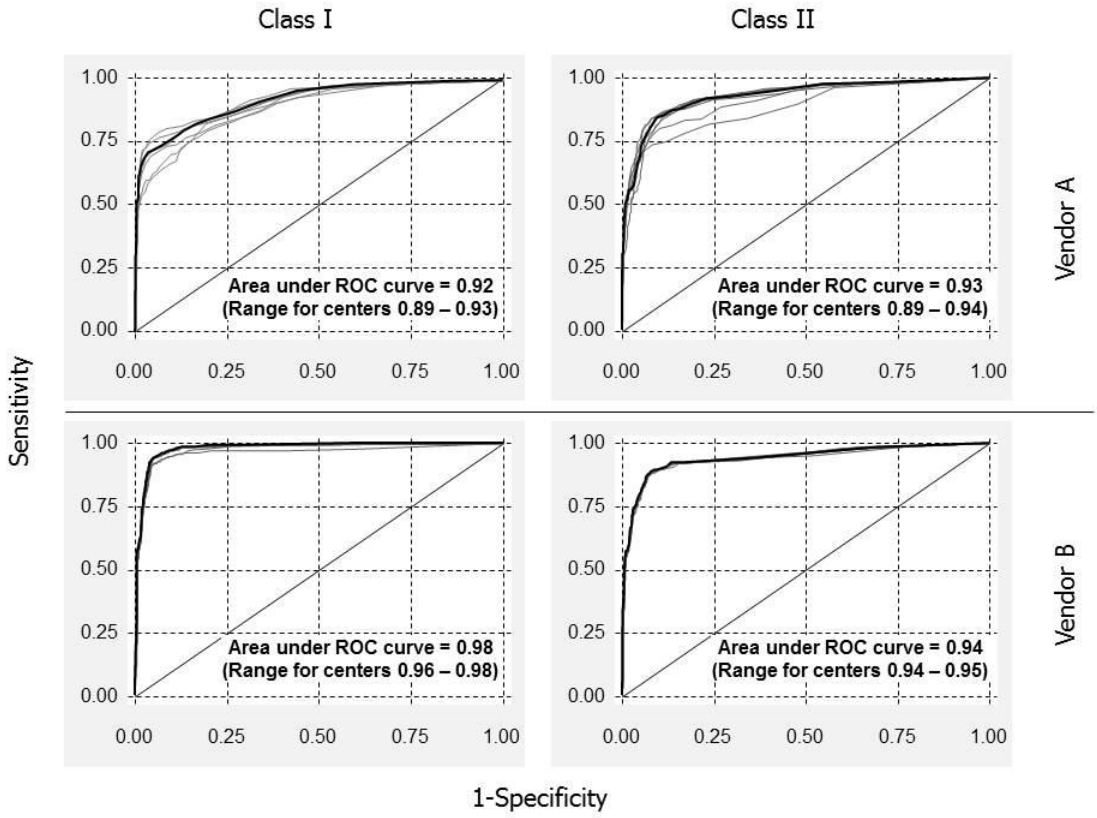
Following adoption of standardized SOPs, reference sera were sent together with identical lots of Luminex reagents to 7 centers across North America. Two single antigen kits (Class I&II) per vendor were analyzed using 14-16 sera samples depending on kit type. For each bead/serum combination (n = 5,236), one vendor's data established truth, then the second vendor's reactions were tested for agreement via receiver operating characteristic (ROC) analysis. Vendor roles were reversed, and the analysis was repeated.

Results:

Figure 1 shows four panels of ROC curves grouped according to kit and vendor. In general, areas under the ROC curves ranged from 0.89 to 0.98 across centers, kits and vendors with standard errors < 0.02. When Vendor B's data established truth, Vendor A's ability to correctly classify reactions was optimized with MFI cutoffs of 1400 units, yielding sensitivity/specificity/accuracy values of 69%/97%/91% and 75%/94%/90% for Class I and II kits, respectively. When testing Vendor B against Vendor A, the optimal MFI cutoff was 3100 units with sensitivity/specificity/accuracy values of 92%/96%/95% and 74%/97%/92% for Class I and II kits, respectively.

Conclusions:

The differences among the centers were small and inconsequential, and the overall consistency in antibody assignments between vendors was excellent. The study represents an important step to validate the reproducibility of the HLA antibody Luminex results.


Class I
Class II
Vendor A
Vendor B
Sensitivity
1-Specificity
1.00
0.75
0.50
0.25
0.00
0.00
0.25
0.50
0.75
1.00
Area under ROC curve = 0.92
(Range for centers 0.89 – 0.93)
Area under ROC curve = 0.93
(Range for centers 0.89 – 0.94)
Area under ROC curve = 0.98
(Range for centers 0.96 – 0.98)
Area under ROC curve = 0.94
(Range for centers 0.94 – 0.95)