A Robust AUC-Based Method for Eplet Calling in SAB Assays

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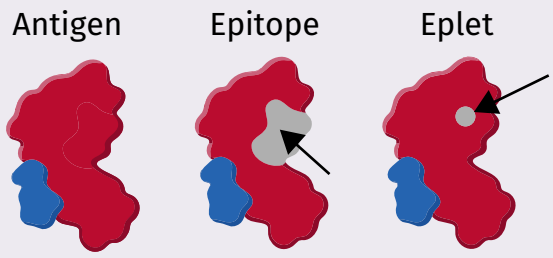
AIM

1) Develop a robust methodology for eplet calling in SAB assays that reduces variability associated with single mean fluorescent intensity (MFI) cutoffs.

2) Apply an area-under-the-curve (AUC)–based approach to cohort-level summarization.

Introduction



An HLA eplet is a small cluster of polymorphic amino acids on the surface of an HLA molecule that represents the functional unit recognized by antibodies. While conceptually powerful for explaining alloantibody specificity, assigning eplet reactivity based on SAB assays is challenging. SAB signals can be influenced by variability in binding affinity, differential eplet density across alleles, and cross-reactivity between structurally related eplets. As a result, distinguishing true eplet-specific antibody binding from assay artifacts remains an area of ongoing difficulty in clinical practice and research.

REFERENCES

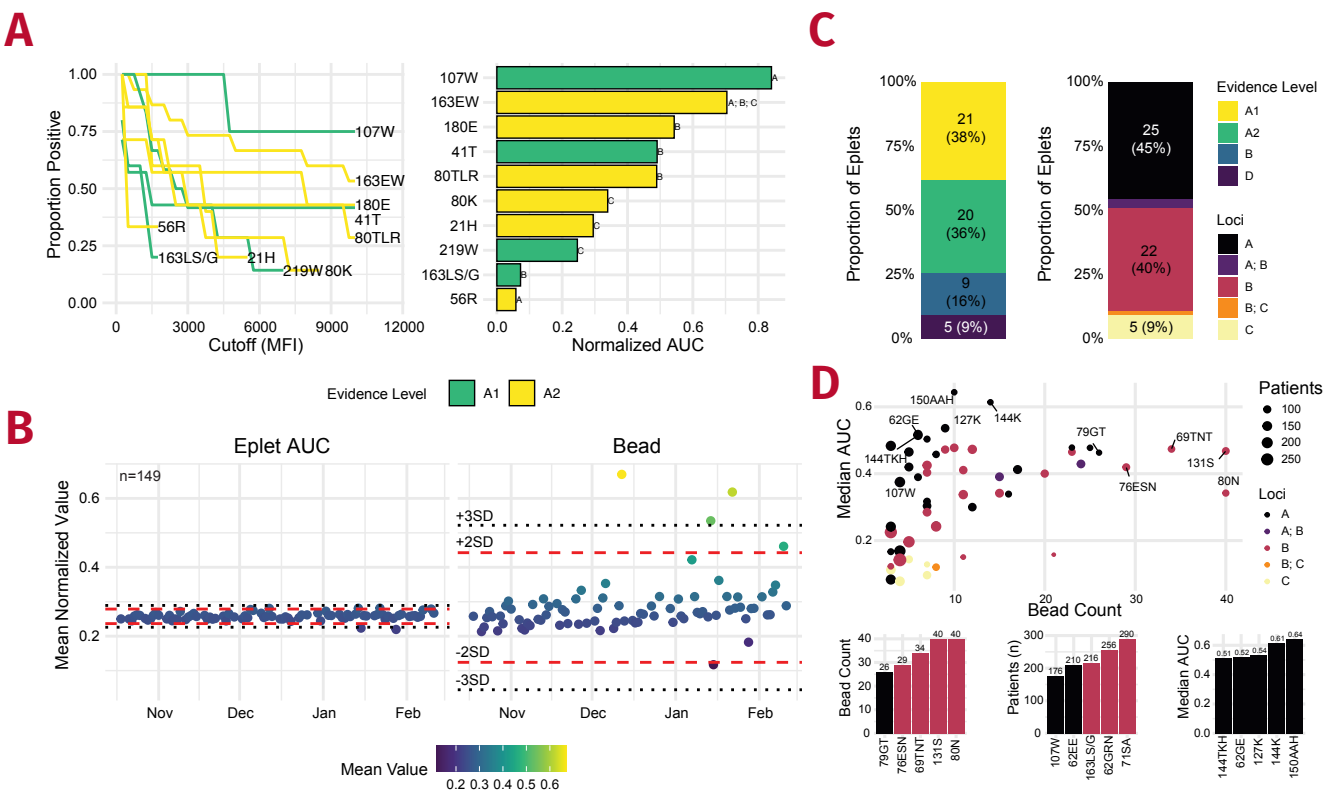
1. Bezstarosti, Suzanne, et al. A comprehensive evaluation of the antibody-verified status of eplets listed in the HLA epitope registry. Frontiers in immunology 12, 2002.

2. Duquesnoy RJ, et al.16th IHIW: a website for the antibody-defined HLA epitope registry. Int J. Immunogenetics, 40: 54-59, 2013

Methodology

We extracted and filtered class I SAB assay (One Lambda, LABScreen) data collected over four months, using a single reagent lot and selecting the latest sample for each patient to avoid duplicates. A total of 1,694 individuals from multiple transplant programs were included. For each bead, the associated allele and eplets were identified using the HLA Eplet Registry, restricting analysis to those with any level of evidence (A1, A2, B, D). At the patient–eplet level, an AUC metric was calculated by taking the derivative of MFI values from 250 to 10,000 and normalizing using 10,000 as a denominator. We compared mean normalized AUC values with mean min–max normalized MFI in a positive control sample (n=149 runs) to assess inter-run variability. We further applied a threshold requiring at least three beads with the respective eplet and ≥0.75 proportion of positive beads across the MFI range to analyze cohort-level eplet patterns.

Results



(**A**) Representative AUC plot derived from MFI values across multiple beads in the class I SAB panel using a positive control sample, accompanied by a bar chart displaying the top eplets by normalized AUC value. (**B**) Comparison of inter-assay variability between the mean normalized AUC method (left panel) and mean min–max normalization of bead-level MFI (right panel) across 148 positive control samples. (**C**) Summary of the confidently mapped (n=55) by evidence level and by locus. (**D**) Confidently mapped eplets across the cohort with median AUC per eplet and the number of beads in the assay containing the eplet with accompanying top 5 eplets by number of beads, number of patients and median AUC.

- Among 1,694 patients, 1,289 (76%) had at least one eplet with a normalized AUC above baseline, and all 67 Class I eplets (A1, A2, B, D) exhibited reactivity within the cohort. After applying more stringent filters, we confidently associated 55 eplets with conventional MFI-based evaluation (**C**).
- The top five eplets most frequently detected in patients, 71SA, 62GRN, 163LS/G, 62EE, and 107W, had lower representation on the panel, as did the five eplets with the highest median AUC values (150AAH, 144K, 127K, 62GE, and 144TKH) (**D**).

CONCLUSION

Our AUC-based approach for eplet calling in HLA SAB assays reduces inter-assay variability and captures eplet reactivity patterns that may be unappreciated with a single MFI cutoff. By integrating MFI values across a dynamic range, this method offers enhanced sensitivity for detecting eplets, even when bead representation is limited. These findings may facilitate more accurate characterization of anti-HLA antibody responses and improve the precision of histocompatibility assessments. Future work will focus on validating these results across larger cohorts and exploring clinical correlations.

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