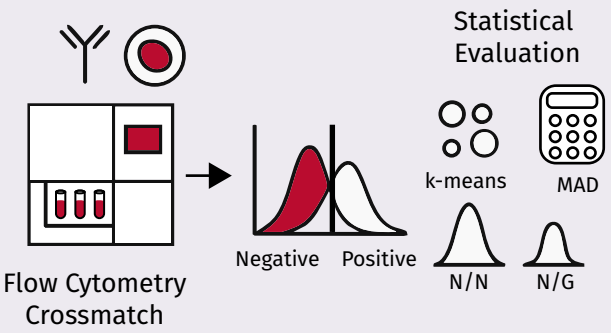
Developing Flow Cytometry Crossmatch Thresholds: A Statistical Evaluation for Clinical Crossmatching Data

Nick Borcherding, Andres Dajles, Gregory Martens, David Clark, Michiko Taniguchi, Chang Liu
Department of Pathology and Immunology, Washington University St Louis

AIM

To assess alternative statistical methods for establishing robust flow cytometry crossmatch thresholds and to evaluate their clinical utility in HLA testing. Our goal is to refine positive/negative discrimination in crossmatch assays and to provide a secondary, data-driven validation tool that supports continuous monitoring of laboratory performance.

Introduction



Flow cytometry crossmatch (FCXM) testing plays a critical role in transplantation by detecting donor-specific antibodies that may cause graft rejection. However, defining cutoff values for FCXM remains challenging because results are influenced by skewed distributions, non-HLA reactivity, and inter-laboratory variability. These factors complicate the distinction between positive and negative results, underscoring the need for robust, data-driven statistical approaches to establish and maintain reliable thresholds.

REFERENCES

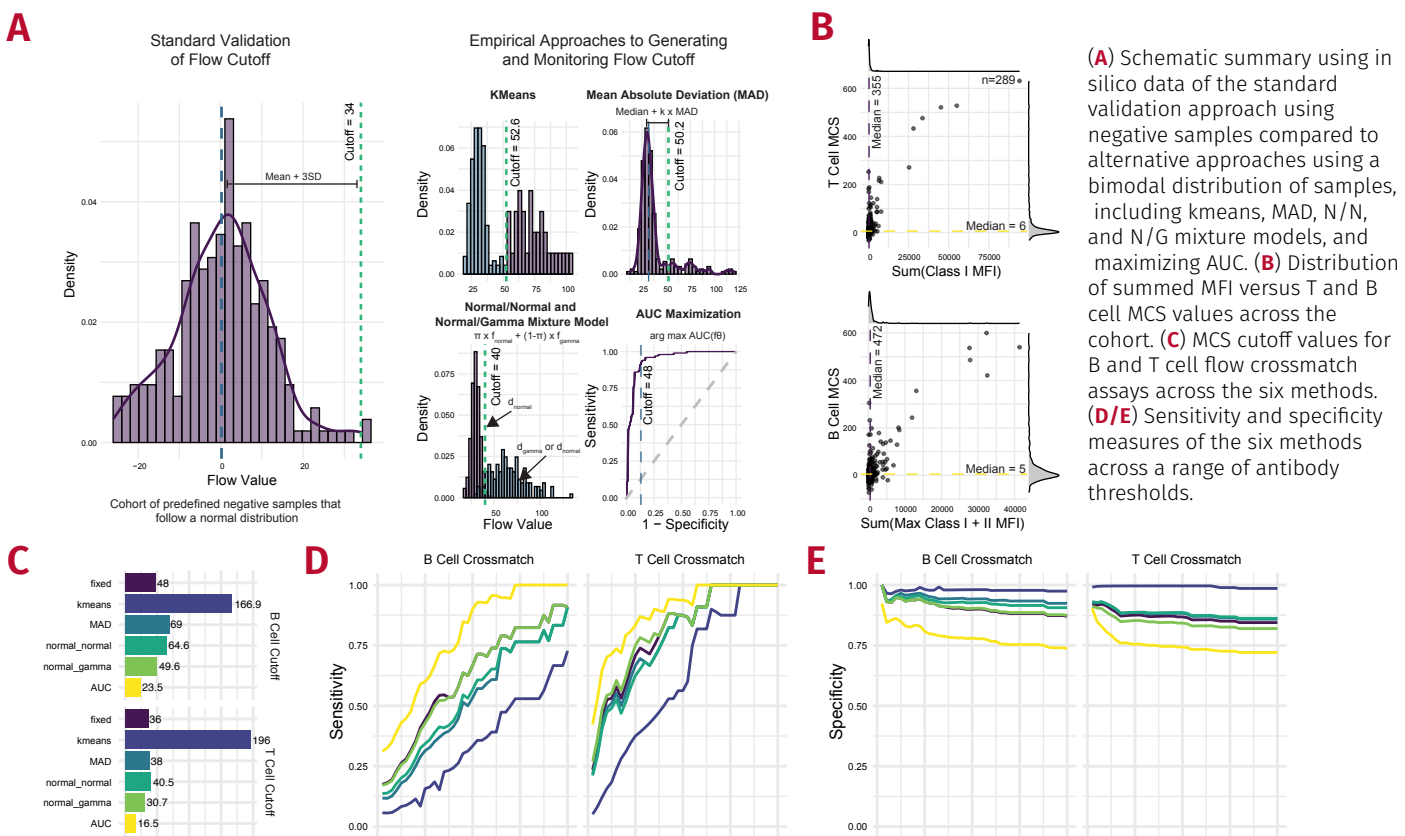
1. Liwski, Robert S., et al. Rapid optimized flow cytometric crossmatch (FCXM) assays: The Halifax and Halifaster protocols. Human immunology 79.1: 28-38, 2018.

2. Couzi, Lionel, et al. Interpretation of positive flow cytometric crossmatch in the era of the single-antigen bead assay. Transplantation 91.5: 527-535, 2011.

Methodology

We analyzed data from 289 patients collected over six months with parallel single antigen bead (SAB) assays and flow cytometry crossmatch testing. Class I mean fluorescence intensities (MFIs) for T cell comparisons were summed, while B cell incorporated the summation of class I and class II MFIs. Conventional laboratory thresholds based on negative samples - median channel shift (MCS) of 36 for T cells and 48 for B cells - were compared with cutoffs derived from several statistical methods, including k-means clustering, median absolute deviation (MAD), a normal-normal (N/N) mixture model, and a normal-gamma (N/G) mixture model. These methods assume a bimodal distribution of MCS values representing positive and negative samples. Additionally, an area-under-the-curve (AUC) approach was employed to integrate MCS and MFI data across a dynamic range (250–10,000 MFI), optimizing sensitivity and specificity.

Results



(**A**) Schematic summary using in silico data of the standard validation approach using negative samples compared to alternative approaches using a bimodal distribution of samples, including kmeans, MAD, N/N, and N/G mixture models, and maximizing AUC. (**B**) Distribution of summed MFI versus T and B cell MCS values across the cohort. (**C**) MCS cutoff values for B and T cell flow crossmatch assays across the six methods. (**D/E**) Sensitivity and specificity measures of the six methods across a range of antibody thresholds.

- Using conventional laboratory thresholds (MCS ≥ 36 for T cells and MCS ≥ 48 for B cells), we identified 50 and 46 positive samples, with 31 patients positive in both assays. Recognizing that crossmatch data of clinical samples are inherently skewed and do not follow a normal distribution, we applied alternative statistical methods that assume a bimodal distribution.
- Both the MAD and mixture models yielded thresholds similar to the conventional cutoffs (**C**). Notably, the N/G mixture model produced cutoffs of 30.7 and 49.6 for T and B cells (**C**), respectively - values within one standard deviation of the laboratory thresholds - and maintained comparable sensitivity and specificity across a broad MFI range (**D,E**).

CONCLUSION

Applying common statistical clustering methodology, including MAD and mixture models, provides a robust framework for determining flow cytometry crossmatch thresholds in real-world HLA testing. Of note, the N/G mixture modeling approach matches the inherent distribution of flow MCS data and produces cutoffs with similar performance to the laboratory validation-based approach. These techniques offer independent verification of previously validated cutoffs and support ongoing laboratory monitoring, ensuring assay consistency over time. Although these methods do not resolve issues such as non-HLA false positives, integrating multimodal modeling strategies could further enhance crossmatch thresholding and improve the reliability of immunological assessments in solid organ transplantation.

WashU Medicine
Washington University St Louis
660. S Euclid Ave., St. Louis, MO 63110
borcherding.n@wustl.edu
cliu32@wustl.edu

Find out more about our research: