

Verification of Flow Cytometry Crossmatch within the Framework of CLSI H62 and ICCS Q&S Module 25

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Abstract

Objective: Flow cytometry crossmatch (FCXM) is a highly sensitive assay for detecting donor-specific antibodies (DSA) in solid organ transplantation. International standards such as ASHI and EFI mandate the verification of performance characteristics before routine clinical use. This study aims to present a practical and guideline-compliant verification roadmap for tissue typing laboratories by re-analyzing routine data to minimize additional workload and kit costs.

Materials and Methods: In accordance with CLSI H62 and ICCS (Module 25) guidelines, three core performance characteristics were evaluated: accuracy, intra-assay precision, and cut-off verification. Pooled negative and positive control sera were utilized. For accuracy, routine FCXM results (2024–2025) were retrospectively compared with virtual crossmatch (VXM) results based on single antigen bead (SAB) assays (MFI >1000). Intra-assay precision was assessed using negative, weak positive, and strong positive controls on a flow cytometer. The internal 95% operational upper limit (OUL95) was calculated using the non-parametric nearest-rank p95 method from negative control distributions (T-cell n=214, B-cell n=211).

Results: FCXM demonstrated 100% specificity for both T and B cells and an overall accuracy of 91% for T cells and 85% for B cells. Sensitivity was 83% for T cells and 71% for B cells. Precision data showed coefficient of variation (%CV) values ranging from 0.7% to 13.5%, confirming suitable reproducibility for clinical use. The laboratory-specific OUL95 values for median IgG-FITC were determined as 190 for T cells and 426 for B cells. Laboratory-derived OUL95 values were fully compatible with routine cut-off criteria.

Conclusion: The FCXM method successfully met the verification requirements of CLSI H62 and ICCS guidelines. The study demonstrates that high specificity and robust intra-assay precision can be achieved with a minimal verification set, providing a reliable and cost-effective model for clinical laboratories.

Keywords: Donor-specific antibodies; flow cytometry; HLA; method verification; transplantation; virtual crossmatch

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