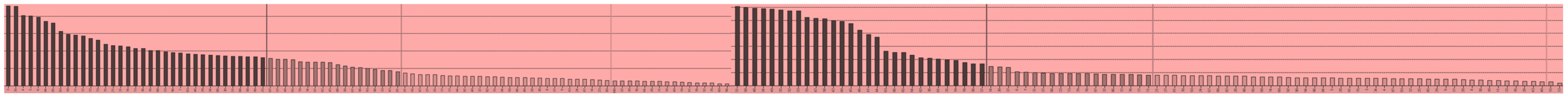
Intra-laboratory MFI Variability in Single Antigen Beads Across Multiple Platforms

While automation clearly reduced operator-driven variability, residual inconsistencies were still observed, influenced by factors such as environmental conditions.



INTRODUCTION: Understanding intra-laboratory test variability is critical in clinical and research settings, as it impacts the reproducibility and interpretation of test results. Intra-laboratory variability can exceed 50% for single antigen bead (SAB) analysis secondary to operator technique or environmental factors like temperatures or pH. Interpretation of fluctuations in bead-specific mean fluorescent intensities (MFI) can be further muddled when factoring in differing assay vendors, antigen sources, and test reagents. While existing literature on variability in HLA SAB studies predominantly focuses on manual techniques, this study explores intra-laboratory variability in the settings of both manual and robotic-assisted workflows.

METHODS: We evaluated Thermo Fisher (OLI) SAB kit results using “in-house” source positive control sera testing across manual and two robotic platforms (LabXpress and HLAPRO). Additionally, we assessed coefficient of variation (CV) to compare the amount of variation relative to its mean. Werfen LSA Class 1 and Class 2 beads were also evaluated using the same positive control sera with requisite manual workflows.

Average % CV using "in house" Positive Control			
Solid Phase Antibody Kits	**LabXpress**	**HLA PRO**	**Manual**
LS-SA1 lot 15	13.79	17.02	14.14
LS-SA2 lot 16	9.75	17.02	17.65
MICA Lot 10	11.56	10.35	13.91
LSM12 Lot 25	7.22	13.75	20.41

Figure 1.

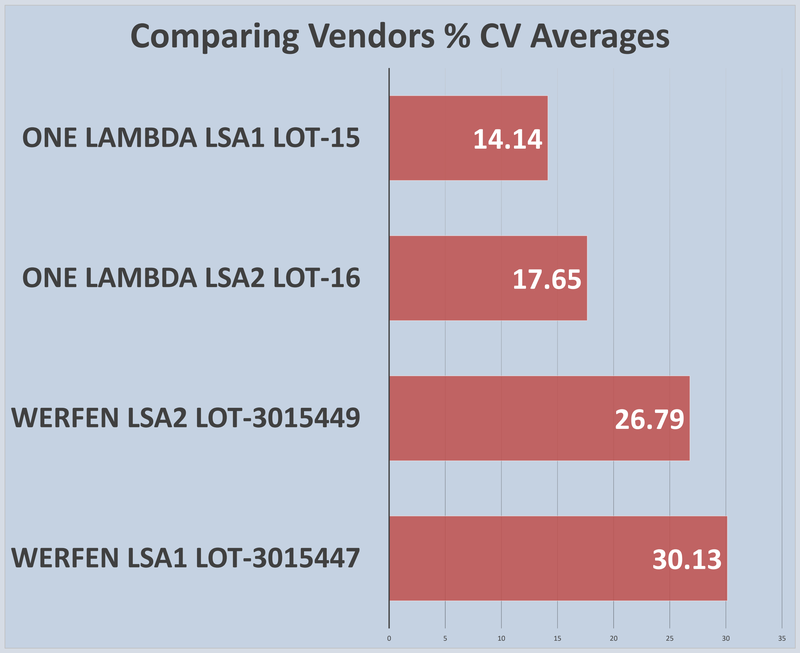


Figure 2.

RESULTS: Coefficients of variation (%CV) were calculated across multiple assay runs. The LabXpress consistently outperformed manual and HLAPRO workflows in precision, with average %CVs of 10.58% (LabXpress), 14.54% (Manual), and 16.53% (HLAPRO). While automation reduced operator-driven variability, residual inconsistencies were still observed (Figure 1). OLI LS SA and Werfen LSA manual methods (spin & flick vs. filter plate) were also compared, revealing less variability in the OLI methods (Figure 2).

CONCLUSION: SAB assays are sensitive to technologist-dependent actions (including pipetting, washing, vortex speeds, centrifuging, etc.), which influence assay performance and the reported MFI value. Our findings demonstrate that despite robotic assistance, significant variability persists. We attribute this residual variability to factors such as instrument calibration, environmental conditions, and sample handling protocols upstream of automation. While comparing vendors, the increase in Werfen LS variability may be attributable to the filter plate method.

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