HLA PRO: Enabling High-Throughput Automated Antibody Screening Workflow

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Abstract

Purpose: Characterization of a fully-automated "walk-away" workflow for performing up to 384 bead-based HLA antibody tests in a single run.

Methods: 4x plates of 96 samples each were run via automation with LABScreen™ mixed-antigen and single-antigen tests on One Lambda HLA PRO pipettor instruments to characterize performance. 1x Plate configured to allow up to 4x secondary antibody types was also tested.

Results: Across 8 runs on two separate instruments, per-plate CV runs positive controls achieved median value of 7.0% (ranging from 5.3-10.9% CV) and 5.61% CV across all runs. Tests achieved 96.8% or greater concordance to manual assay runs.

Introduction

The One Lambda HLA PRO is a pipettor instrument that can enable full "walk-away" automation of HLA testing workflows in one platform (the HLA PRO currently supports both bead-based and NGS-based assays). Key features include:

- Integration with centrifuge and bead-based analyzers (such as the LABScan3D™). Users only need to set up reagents, and the rest is automated.
- Independent 8-channel pipettor (pipetting ranges 1 - 1000 µL) with plate gripper tool.
- Touchscreen interface guides users through set up procedure.
- Interoperability of HLA PRO with One Lambda Fusion and TSV software, allowing integration of experimental design, assay instruction generation, and data analysis.

Figure 1. One Lambda HLA PRO automated pipetting system. The right is a bead-based analyzer, and the left portion houses a pipetting and plate-handling system.



Materials and methods

Automation Workflow

HLA PRO configuration with Bead Analyzer (LABScan3D™) and Hettich™ centrifuge deck was used for automation runs. Barcoded Bead-products were scanned and dispensed from 1.5mL Centrifuge tubes. Plates had 20 µL of serum samples (internal reference set) aliquoted to 96-well full skirted plate and spun down in HLA PRO centrifuge prior to bead dispensing.

Data Analysis

Readout raw files from bead analyzer showing phycoerythrin-based fluorescence intensity and bead counts for each bead-region were extracted and evaluated on the basis of key performance metrics such as concordance to manual runs, inter and intra-assay coefficient of variation.

Multi-plate Runs of Antibody Tests for Reproducibility/Repeatability

Each 4 plate run consists of 2 plates that used the LABScreen™ Mixed Class I & II and 2 plates that tested LABScreen™ Single Antigen HLA Class 2. Users set up the run on two separate instruments, each plate consisting of 96 samples.

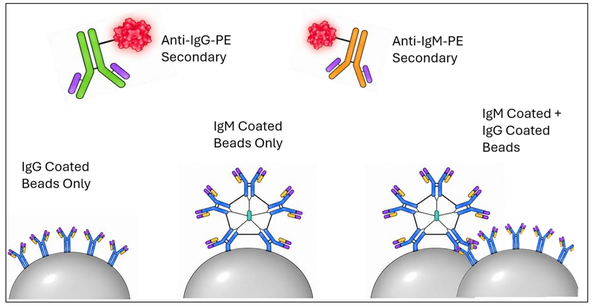
Multi-secondary Runs to Verify Functionality

For testing of multi-secondary antibody performance two different secondary antibodies (IgG and IgM) were tested with control samples that consisted of beads coated with either IgG or IgM antibodies. The different components tested in combination are illustrated in Figure 2. This test provided proof of concept for automated processing of multiple secondary antibody classes.

Manual vs. Automated Runs

As proof of concept, LS2PRA was run on 25 serum samples to compare the HLA PRO runs with a manual run. Trimmed mean signals were correlated with runs on the HLA Pro using different secondary antibody concentrations.

Figure 2. Positive Control Experiment to verify functionality and capability of performing multiple secondaries. IgG and IgM coated positive control beads were tested combinatorially with Anti-IgG-PE and Anti-IgM-PE on the HLA PRO in order to verify the capability of the instrument in assessing immunoglobulin class antibodies in specimens.



Results

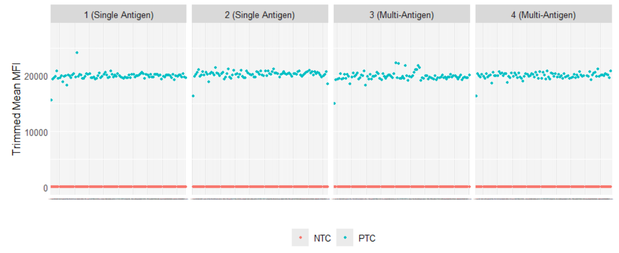
4 Plate Run – 384 Samples Total – Performance Data

Results demonstrated that the HLA PRO was able to achieve performance with all concordance being >96% relative to a manual run (Table 1). Due to the absence/presence of beads that may be positive depending on the presence of antibodies, analysis was also performed on bead regions where the MFI is higher than 1000, showing that in these samples that CV ranged from 3.3% - 7.0%

Table 1. Performance Characterization in 4 Plate Runs

Instrument	Plate #	Product SKU	Concordance	Average CV%	Average %CV MFI ≥ 1,000
A	1	LS2A01	96.9%	5.8%	3.3%
	2	LS2A01	96.9%	10.9%	4.3%
	3	LSM12	100.0%	5.7%	3.4%
	4	LSM12	99.9%	5.3%	3.6%
B	1	LS2A01	96.9%	8.3%	6.0%
	2	LS2A01	96.8%	8.3%	7.0%
	3	LSM12	100.0%	7.8%	6.7%
	4	LSM12	100.0%	6.2%	5.2%

Figure 3. Representative visualization data from 4 plate run on instrument B, showing measured trimmed mean MFI on each of the 96 wells for positive and negative controls on each plate of the 4 plate run.



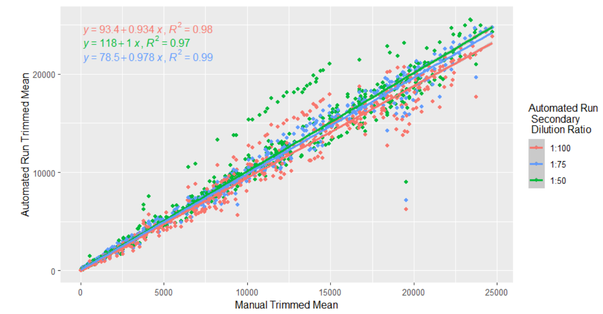
The HLA PRO automation scheduler dynamically coordinates between necessary assay steps including liquid dispensing, bead mixing, plate centrifugation, supernatant removal, shaking, and loading the plate in the FM3D. Runs are blocked into runs of 2 plates each, with approximate times of each run detailed in Table 2. Run length is also scaled appropriately based on the samples present on each plate.

Table 2. Time for different quantities of runs on HLA PRO

Study	Plate	# Samples	Run Time
4 Plate	1	96	3hr 54 min
	2	96	5hr 16 min
	3	96	7hr 49 min
	4	96	8hr 33 min

HLA PRO Automated vs. Manual Runs of 25 Serum Samples

Figure 4. Correlation of trimmed means on manual vs. automated runs

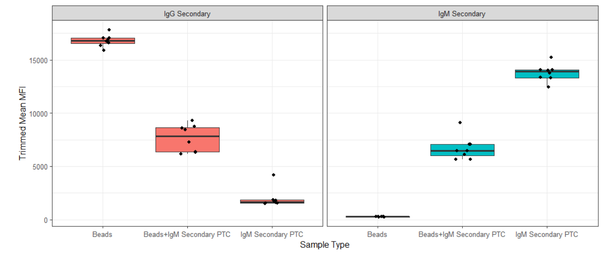


Comparison studies showed high correlation between automated and manual runs.

Automated Runs with Multiple Secondary Antibodies

Tests involving the usage of IgG and IgM secondary antibodies in combination with mixtures of IgG and IgM positive control bead material showed that generally predictable reactivity patterns (see Figure 5) of reactivity. Mixture of the beads shows reasonable signal change patterns.

Figure 5. Fully automated run of IgG and IgM secondary antibodies in combination with IgG positive control beads, IgM positive control beads, and combined IgG+IgM positive control beads.



Conclusions

The HLA PRO is a high-performance platform that allows the full walk-away automation of antibody related tests for HLA Antibody screening of up to 384 samples.

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