Enhancing Flow Cytometry Workflows: Evaluating the Curiox C-FREE™ Pluto System for Whole Blood Immunophenotyping Sample Processing

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Overview

Purpose: To demonstrate the performance and benefits of the Curiox C-FREE™ Pluto System (Pluto) in automating whole blood surface and intranuclear staining workflows for flow cytometry.

Methods: Fully-automated reagent and buffer preparation, including antibody cocktail preparation (Ab cocktail prep), whole blood staining, lysis and fixation were performed on Pluto, with a side-by-side comparison against manual sample processing via centrifugation.

Results:

- Enhanced cell retention and population resolution with Pluto.
- Comparable population and subpopulation frequencies between Pluto and manual method.
- Overall reduction in hands-on time with Pluto, improving workflow efficiency and consistency.

Introduction

Flow cytometry remains a critical tool for immunological research and diagnostics, yet traditional manual workflows can be labor-intensive and inconsistent. The Curiox C-FREE™ Pluto System (Pluto) introduces a fully-automated workflow solution, including Ab cocktail prep, seamlessly integrating with conventional protocols without requiring adaptation.

Pluto incorporates gentle washing of samples as opposed to harsh centrifugal forces with traditional methods. This reduces mechanical stress to cells, while removing debris and unbound antibodies in the supernatant. Complete automation eliminates manual intervention, reducing errors and ensuring consistent reproducibility across donors and replicates. By maintaining sample integrity and enhancing workflow precision, Pluto optimizes laboratory productivity and data reliability.

This poster aims to present the capabilities of Pluto in whole blood immunophenotyping compared to traditional centrifugation. In partnership with a large Contract Research Organization (CRO) actively pursuing automation strategies, we demonstrated improvement in data quality and time-savings with Pluto.

Methods

A side-by-side comparison of whole blood surface and intranuclear staining procedure was performed with Pluto vs manual method (Figure 1). Reagent and buffer preparation (Figure 1B), including Ab cocktail prep (Figure 1A), are fully-automated on Pluto, reducing errors through manual pipetting. Peripheral whole blood was obtained from healthy human donors, stained with surface antibody cocktail, lysed and washed via Pluto or manual method. For surface staining only, samples are collected for flow cytometry analysis. Otherwise, samples undergo fixation and permeabilization, followed by intracellular staining. Counting beads are added prior to flow cytometry acquisition for enumeration of absolute cell number.

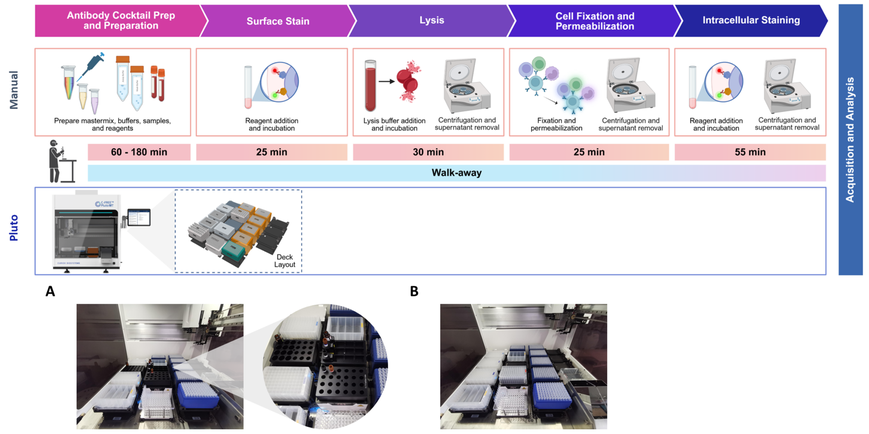

Figure 1. Schematic diagram showing a time comparison study of whole blood surface and intranuclear staining between Pluto and manual method. Seamless method transfer from traditional centrifugation to Pluto eliminates the need for adaptation. Pluto provides end-to-end automation from Ab cocktail and buffer prep to sample processing for downstream analysis, significantly reducing hands-on time and overall improving workflow efficiency. **A.** Ab cocktail prep is customizable with interchangeable adaptors that fit different antibody vials of various sizes and volumes to suit assay needs. **B.** Lysis buffer and wash buffer are pre-prepared in 4-slot reservoirs and Pluto performs automated buffer prep by pipetting required buffer volumes into respective 96-well plates.

Results

Pluto offers walk-away, high-accuracy solution for Ab cocktail prep

While total run time for Ab cocktail prep on Pluto is longer (Figure 2A) compared to manual prep, the system offers a complete walk-away solution, allowing researchers to focus on other tasks. Additionally, manual prep of antibody mastermixes is prone to pipetting errors and operator-to-operator variability. Equipped with Liquid Level Detection (LLD), Ab cocktail prep on Pluto ensures high accuracy and reproducibility–critical for high-parameter panels where low error rates and standardization are essential to reliable laboratory workflows. When comparing whole blood immunophenotyping using Ab cocktail prep on Pluto vs manual prep, stain indices of cell markers are similar between methods (Figure 2B), demonstrating consistent staining performance with Pluto. Overall, the long-term benefits of Ab cocktail prep with an automated system such as Pluto far outweigh the slight time increase.

A

10-color panel		
	Manual prep	Ab cocktail prep
Hands-on time	4 min 14 sec	0 min
Hands-off time	0 min	10 min 17 sec
24-color panel		
	Manual prep	Ab cocktail prep
Hands-on time	9 min 58 sec	0 min
Hands-off time	0 min	16 min 45 sec

B

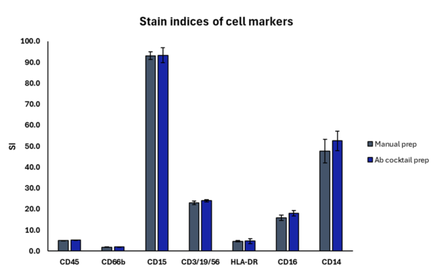

Figure 2. Ab cocktail prep on Pluto provides long-term benefits over manual prep. A. Time comparison of Ab cocktail prep for 10-color and 24-color panels on Pluto vs manual prep (n=10 tests). Manual prep time is averaged across 2 operators. **B.** Comparable stain indices of cell markers with whole blood immunophenotyping using Ab cocktail prep on Pluto vs manual prep. Error bars indicate standard deviation of technical replicates (n=3).

Results

Enhanced cell retention and population resolution

Cell loss is incurred with traditional methods using centrifugation. Harsh centrifugal forces cause mechanical stress to cells, impacting downstream analyses. Cells are better retained with Pluto (Figure 3B) as its gentle washing minimize turbulence to settled cells. Additionally, efficient washing on Pluto removes debris and reduces background, resulting in improved data quality. Lymphocytes are better characterized, with clear separation between positive and negative populations (Figure 3A). Low background on intranuclear Ki-67 staining is also notable with Pluto compared to manual method (Figure 3C).

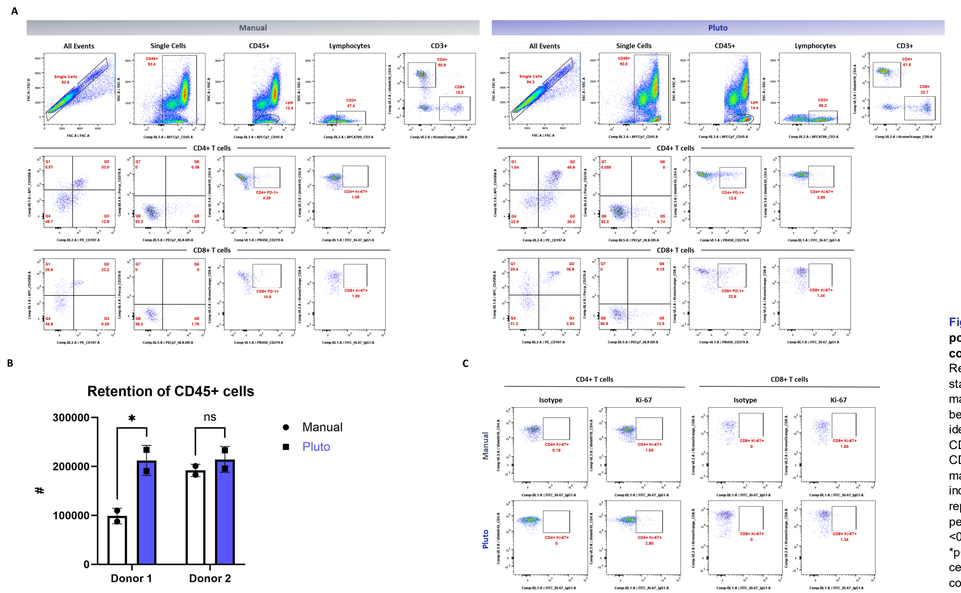

Figure 3. Enhanced cell retention and population resolution with Pluto compared to manual method. A. Representative scatterplots for intranuclear staining on resting whole blood with Pluto vs manual method. T cell populations are better-resolved with Pluto, with clear identification of memory subsets (CD197 vs CD45RA). **B.** Higher absolute number of CD45+ cells retained with Pluto compared to manual method across 2 donors. Error bars indicate standard deviation of technical replicates (n=2). Statical analysis was performed using T-test, and a p-value of <0.05 denotes statistical significance. *p<0.05. **C.** Intranuclear Ki-67 staining on T cells with Pluto shows low background compared to manual method.

Comparable population and subpopulation frequencies

Population frequencies are similar between Pluto and manual method in whole blood immunophenotyping (Figure 4B), with comparable lysis efficiency (%CD45+) (Figure 4A).

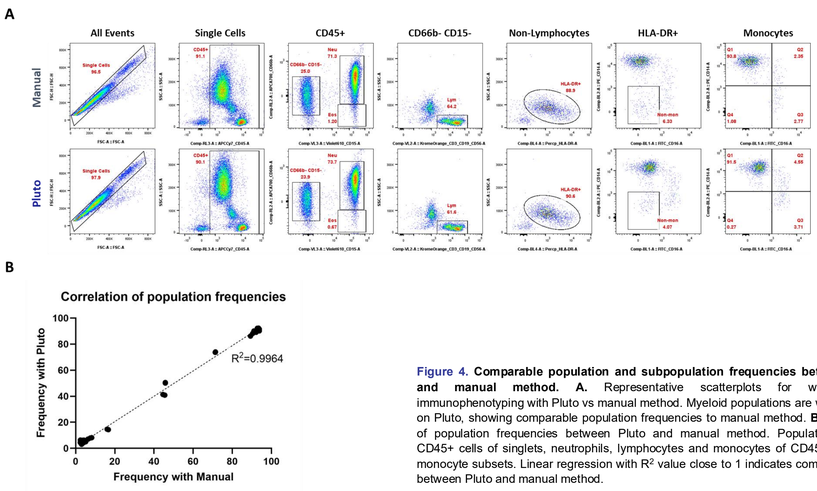

Figure 4. Comparable population and subpopulation frequencies between Pluto and manual method. A. Representative scatterplots for whole blood immunophenotyping with Pluto vs manual method. Myeloid populations are well-resolved on Pluto, showing comparable population frequencies to manual method. **B.** Correlation of population frequencies between Pluto and manual method. Populations include CD45+ cells of singlets, neutrophils, lymphocytes and monocytes of CD45+ cells, and monocyte subsets. Linear regression with R^2 value close to 1 indicates comparable data between Pluto and manual method.

Conclusion

Pluto demonstrates benefits in end-to-end automation for sample preparation, with Ab cocktail prep significantly reducing manual errors and improving workflow efficiency. This innovative system represents a paradigm shift in flow cytometry sample processing, driving advancements in diagnostic and research workflows while ensuring high-quality, reproducible results.