Enhancing Immunophenotyping Precision: A Centrifuge-Less Approach with Curiox C-FREE™ Pluto LT System for Automated Whole Blood Sample Preparation

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Introduction

Immunophenotyping is crucial for analyzing cellular markers to understand diseases and evaluate treatments. Flow cytometry, vital for detecting rare cell populations in complex samples like whole blood, requires preserving sample heterogeneity until analysis. The Curiox C-FREE™ Pluto LT system (Pluto LT system) provides a high-throughput, centrifuge-free solution for maintaining blood sample integrity. This automated system streamlines staining and lysis, ensuring reproducibility with a minimal laboratory footprint.

- **Objective:** Compare Pluto LT automation with traditional centrifugation techniques to identify T-cell subsets in human whole blood via flow cytometry.
- **Evaluation:** Evaluated stain indices of CD markers, consistency of population frequencies, and retention rates of cell subpopulations following lyse-wash-stain-wash or stain-lyse wash whole blood sample preparation
- **Results:** The Pluto LT wash process retained lymphocytes, Treg cells, and memory cell subsets effectively , showing better debris removal than seen with centrifuge. Collaborative validation from Charles River Laboratories confirmed the reliability and reproducibility of this method with fresh and aged human whole blood.

Study Design

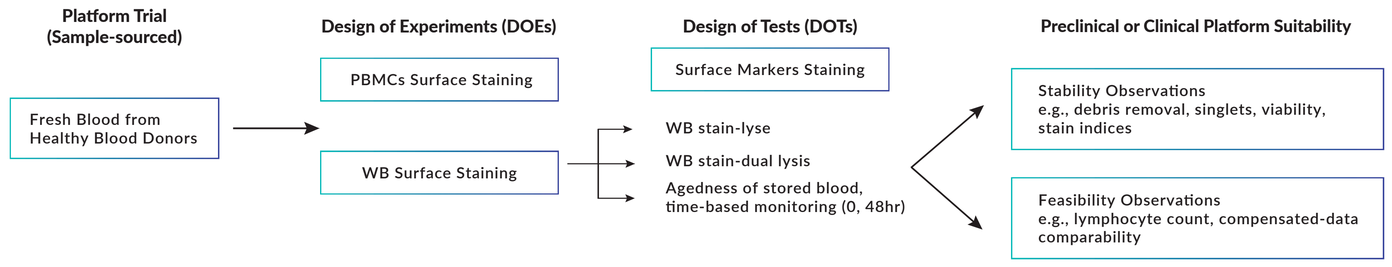

Fig 1. The Pluto LT system efficiently processes whole blood samples (up to 50 µL) for flow cytometry analysis. Healthy donor blood samples and cryopreserved PBMCs were evaluated using surface staining protocols to observe the stability and feasibility of the Pluto LT system, aiming to establish its standardization and scalability.

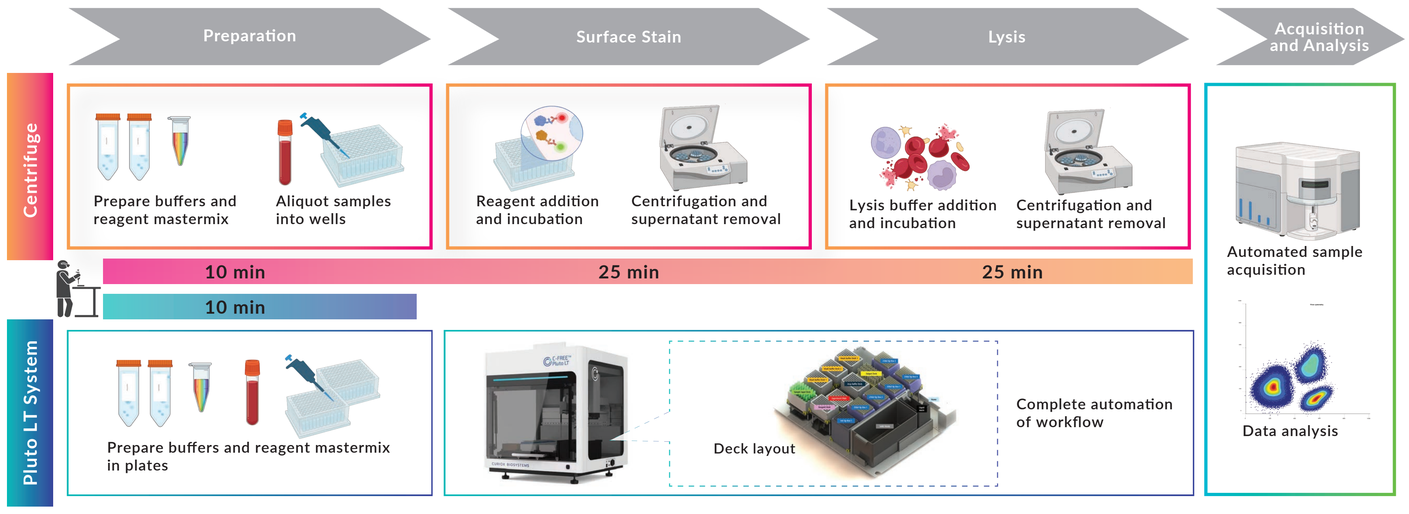

Fig 2. General approach for flow cytometry whole blood/sample preparation with conjugated primary antibodies and/or indirect staining customized for the Pluto LT system, incorporating direct adaptations from conventional centrifuge-washing methods to Pluto LT washing. This results in reduced hands-on time, facilitating a more efficient workflow and reduced variabilities.

Marker	Clone	T-cell Panel
Viability stain	-	BV510
CD3	UCHT1	APC
CD4	SK3	PerCP-Cy5.5
CD27	O323	BV650
CD45	HI30	FITC
CD45RA	HI100	APC/Fire 750
CD127	A019D5	PE-Cy7
CD25	M-A251	PE

Marker	Clone	Basic Panel
Viability stain	-	eF780
CD45	HI30	eF506
CD3	OKT3	SB600
CD4	OKT4	PerCP-Cy5.5
CD8	SK1	PE-Dazzle 594
CD19	HIB19	FITC
CD16+CD56	B73.1 + HCD56	PE
CD11b	ICRF44	BV421
CD11c	Bu15	PE-Cy7

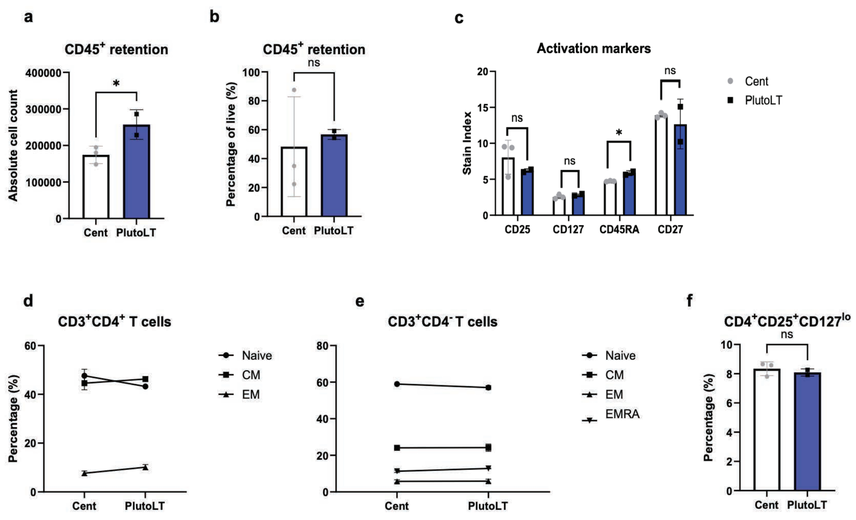

Fig 3. Healthy donor blood sample preparation for flow cytometry immunophenotyping using the Pluto LT system. 50 µL whole blood stained with T-cell panel (viability stain BV510, CD3 (UCHT1) APC, CD4 (SK3) PerCP-Cy5.5, CD27 (O323) BV650, CD45 (HI30) FITC, CD45RA (H100) APC/FIRE750, CD127 (A019D5) PE-Cy7, CD25 (M-A251) PE), lysed before washing using either centrifuge or the Pluto LT system and acquired on a Beckman Coulter CytoFLEX LX . Better debris removal of $CD45^-$ events (a) and $CD45^+$ leukocytes retention (b) was determined with CD45 staining. Stain index on the Pluto LT system is higher for CD45RA and comparable to centrifuge for other activation markers (c). Naïve, CM: central memory, EM: effector memory, EMRA: effector memory subpopulations gated on $CD3^+$ $CD4^+$ T-cells (d) and $CD3^+CD4^-$ T-cells (e) respectively, are comparable for both methods. The Pluto LT system Treg cell frequency is comparable to conventional methods with lower variability (f). Error bars represent standard deviation of technical replicates (PlutoLT: n=2, Cent: n=3). Statistical analysis was performed by Student's t-Test; * <0.05.

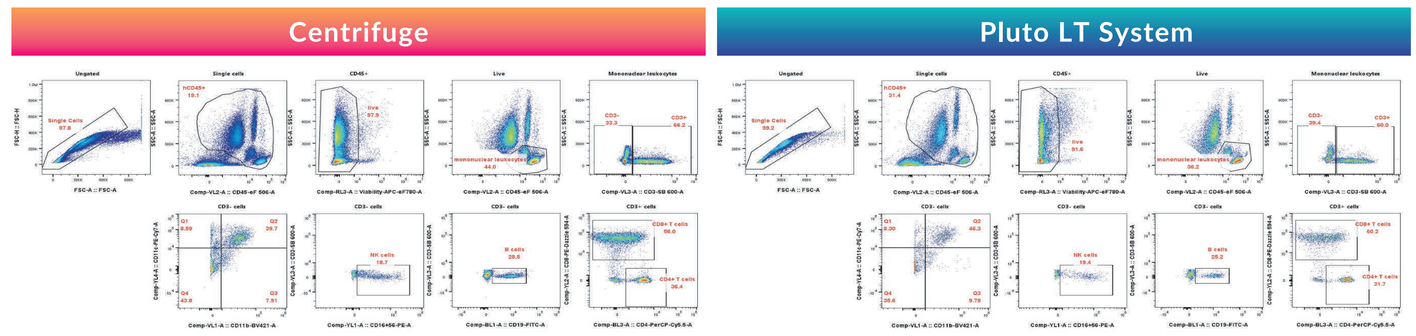

Fig 4. Pluto LT system WB sample preparation for flow cytometry analysis. Representative dot plots of one donor WB (technical replicates n=4) processed with conventional centrifuge method or the Pluto LT system are shown. Lower non-$CD45^+$ events in the Pluto LT system-washed samples indicates better wash efficiency compared to the conventional centrifuge method. Population resolution and frequencies are consistent between both methods. Samples were stained with basic immunophenotyping panel (Viability stain eF780, CD45 (HI30) eF506, CD3 (OKT3) SB600, CD4 (OKT4) PerCP-Cy5.5, CD8 (SK1) PE-Dazzle 594, CD19 (HIB19) FITC, CD16+CD56 (B73.1+HCD56) PE, CD11b (ICRF44) BV421, CD11c (Bu15) PE-Cy7) and acquired at a rate of 100 µL/min on an Invitrogen™ Attune™ NxT Flow Cytometer.

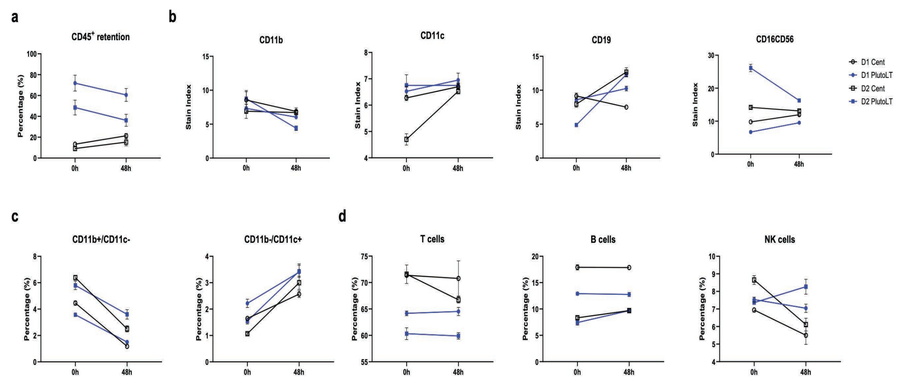

Fig 5. Pluto LT system processed donor blood from two different timepoints. 50 µL WB was stained with TBNKM panel, lysed and washed using the Pluto LT system or conventional centrifuge method. Higher percentage of $CD45^+$ cells in live cells indicates better debris removal by the Pluto LT system (a). Stain indices of immune cell subsets processed by the Pluto LT system are comparable to conventional methods for both timepoints at 0h and 48h (b). Frequencies of myeloid (c) and lymphoid (d) subpopulations were consistent over both timepoints in Pluto LT system samples. Error bars represent standard deviation of technical replicates (n=4).

RBC lysis Buffers	Leukocytes Recovery	Debris Removal	Subpopulation Frequencies
Gibco™ ACK lysing buffer	++	++	+++
BC VersaLyse™	+	+	++
BC FLEXLyse™	+++	+++	+++
BD Pharm Lyse™	++	++	++
BD FACS™ Lysing Solution	+++	+++	+++
STEMCELL™ Ammonium Chloride Solution	+++	+++	+++

Fig 6. Comparison of different commercial buffers used in C-FREE wash methods relative to conventional centrifuge method. Leukocyte recovery (frequency of single cells) from healthy donor blood, debris removal and subpopulation frequencies observed with respective lysis buffers are qualified based on performance of C-FREE method against the conventional method. '+' and '+++' denote least and highest level of equivalence, respectively.

Discussion and Conclusion

The findings presented in this study lead to a compelling discussion about the Pluto LT system's capacity to serve as an advantageous alternative to centrifugation for preparing whole blood samples. It has demonstrated a clear proficiency in removing debris more effectively, particularly with respect to achieving higher live cell leukocyte frequencies. The system's ability to maintain immune cell subpopulation frequencies and stain indices at levels comparable to those achieved by centrifugation is also noteworthy. The system's lower standard deviation in leukocyte and Treg cell frequencies speaks to its enhanced precision and reliability for the rarer subsets. The validation of the Pluto LT system by Charles River Laboratories adds weight to these findings, offering a credible endorsement of the method's consistency and dependability across different settings.

The Pluto LT system's reliable performance with aged blood samples underscores its robustness, further cemented by its ability to save time in the laboratory. However, it's important to acknowledge that while the Pluto LT system shows substantial promise, the retention of CD45+ leukocytes presents an opportunity for further refinement, possibly through the optimization of assay buffers and wash parameters.

In conclusion, the Pluto LT system emerges as a promising tool that could reshape current practices by enhancing the accuracy and repeatability of assays. Its implications for the future of whole blood staining and lysis processes are substantial, indicating a shift towards more automated and standardized immunological assays that could have far-reaching effects on both clinical diagnostics and research methodologies.

Speak to us

Acknowledgements

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