

(frozen at -80 °C)

Comparison of Whole Blood Preservation Methods for Cytometric Analysis

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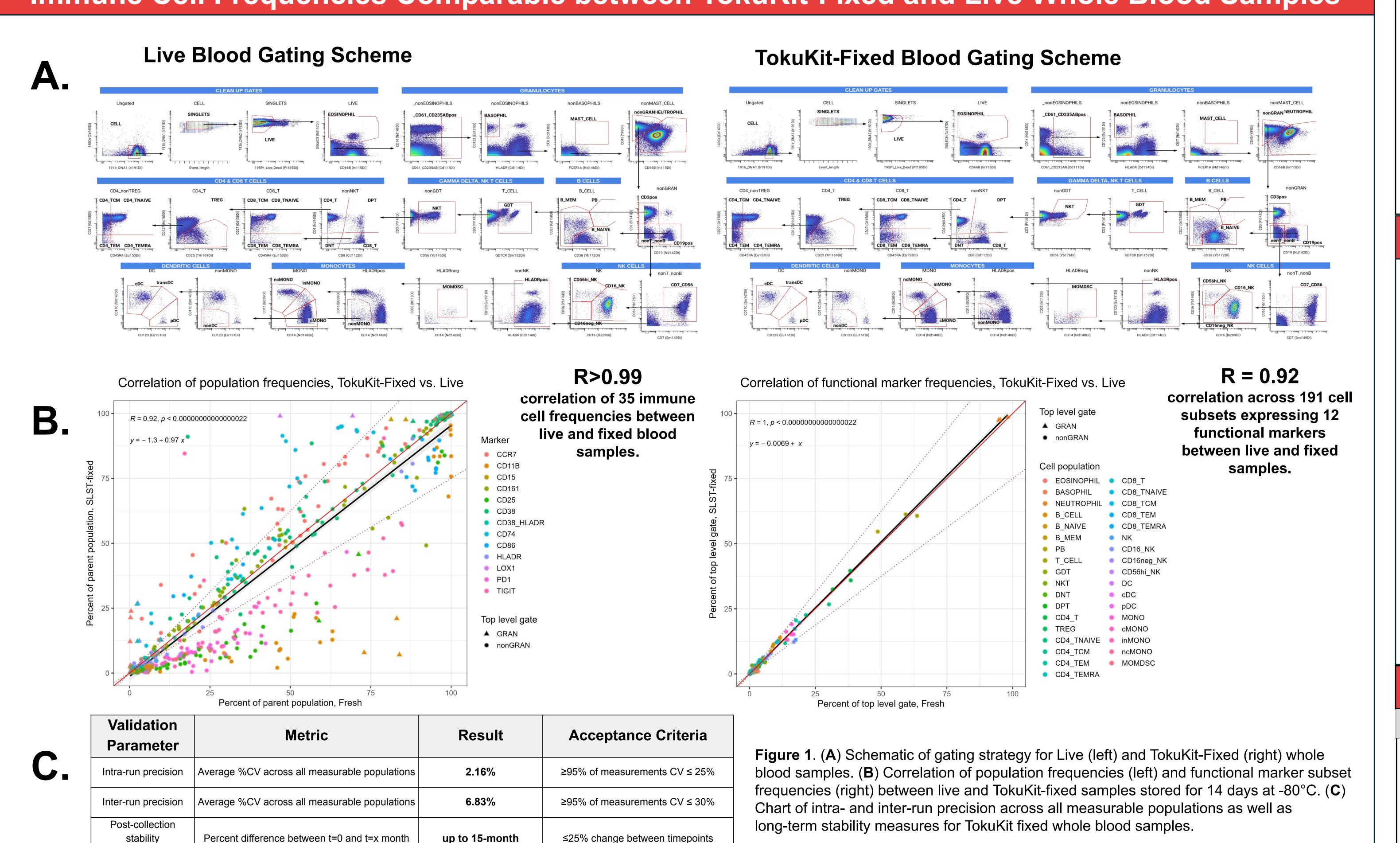
Introduction

Clinical trials rely on high-dimensional immune profiling to evaluate treatment responses, but inconsistencies in blood collection, shipping, and processing result in 20%+ sample loss. Approximately 80% of samples are processed live, yet transport under variable conditions causes a 49% reduction in total cell count with disproportionate loss of monocytes and neutrophils. These challenges highlight the need for reliable, scalable whole blood stabilization methods that preserve immune composition and support both surface and intracellular marker detection.

We compared three blood stabilization methods: Cyto-Chex, TransFix, and CellSave, to Teiko's two-step blood preservation kit (TokuKit). Cyto-Chex supported convenient single-tube collection but resulted in reduced B cell frequencies and increased CD4⁺CD8⁺ T cell artifacts after 7 days. TransFix preserved total population frequencies but showed poor resolution of monocyte subsets (CD14/CD16) and failed to detect intracellular markers, including T-bet. CellSave maintained overall immune frequencies and achieved the highest correlation to TokuKit samples frozen at -80°C controls at 7 days (r = 0.97 with BD Lyse) but exhibited slightly elevated frequencies of granulocytes.

TokuKit samples can be stored at –80°C for more than 15 months and showed strong correlation to fresh samples (R > 0.97). No measurable skewing of immune population frequencies or signal loss was observed over time, and both surface and intracellular markers were reliably detected. These results demonstrate that TokuKit provides robust long-term whole blood stabilization, maintains accurate immune population frequencies, and supports detection of both surface and intracellular markers. This approach enables more flexible sample handling for multi-site clinical trials and retrospective immune monitoring studies.

Immune Cell Frequencies Comparable between TokuKit-Fixed and Live Whole Blood Samples



Methods and Panel for Comparison of Stabilization Methods

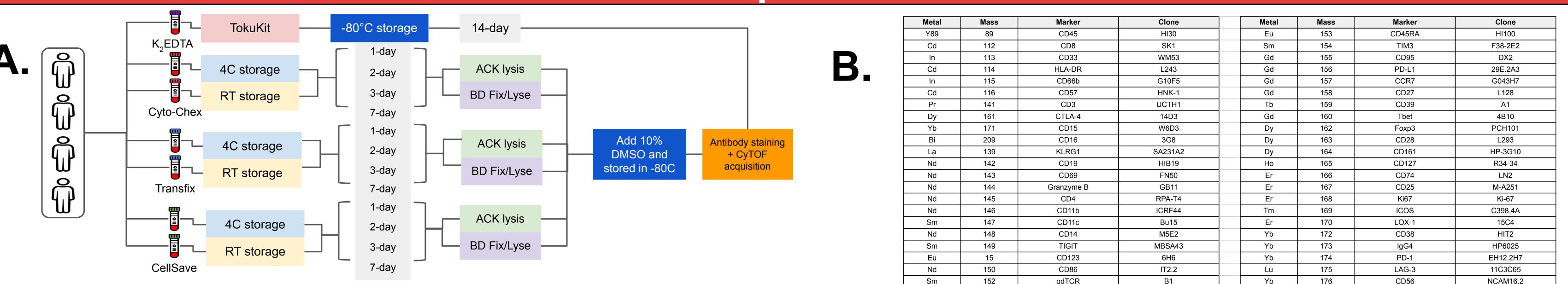
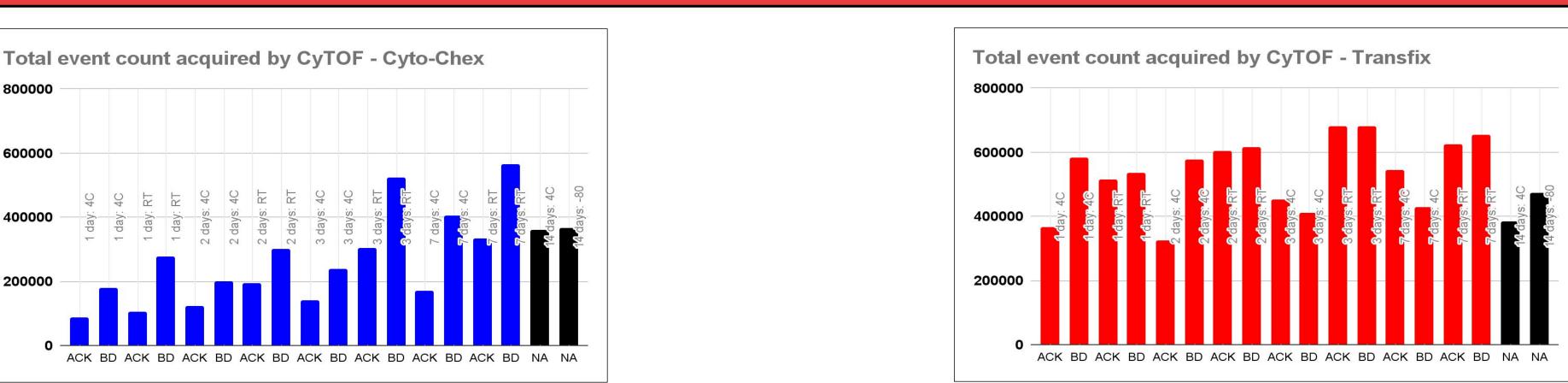


Figure 2. (A) Schematic of collection and acquisition methods. Whole blood from four healthy donors was collected into Cyto-Chex, Transfix, CellSave, or TokuKit (contains buffers "Stable Lyse" and "Stable Store,"(SLST) buffers manufactured by Smart Tube®). TokuKit samples were frozen at -80°C for 14 days and served as the gold standard reference control. Cyto-Chex, Transfix, and CellSave samples were stored at 4C or room temperature (RT) for 1, 2, 3, or 7, days, lysed with ACK or BD Lyse/Fix, and stained with (B) a 41-marker mass cytometry immune profiling panel, and acquired on a Helios mass cytometer.

Transfix Preserves Total Event Counts During Acquisition



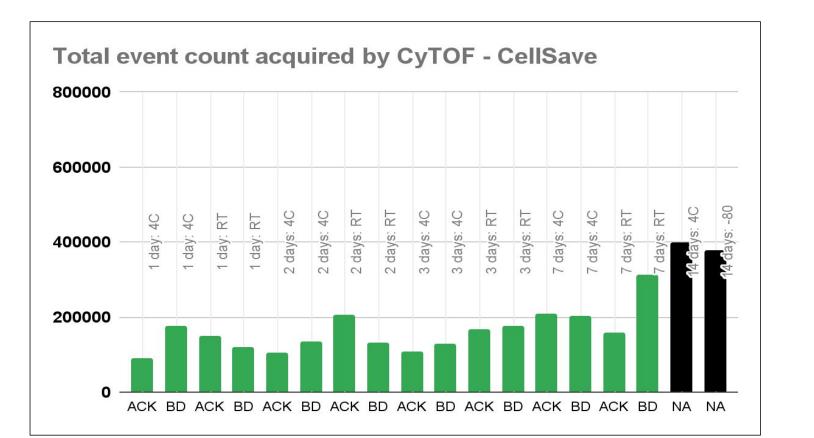


Figure 3. Comparison of total events acquired by CyTOF after blood collection in Cyto-Chex (blue), Transfix (red), and CellSave (green) compared to TokuKit (black) from Donor 1.

Comparison of Whole Blood Preservation Methods to TokuKit

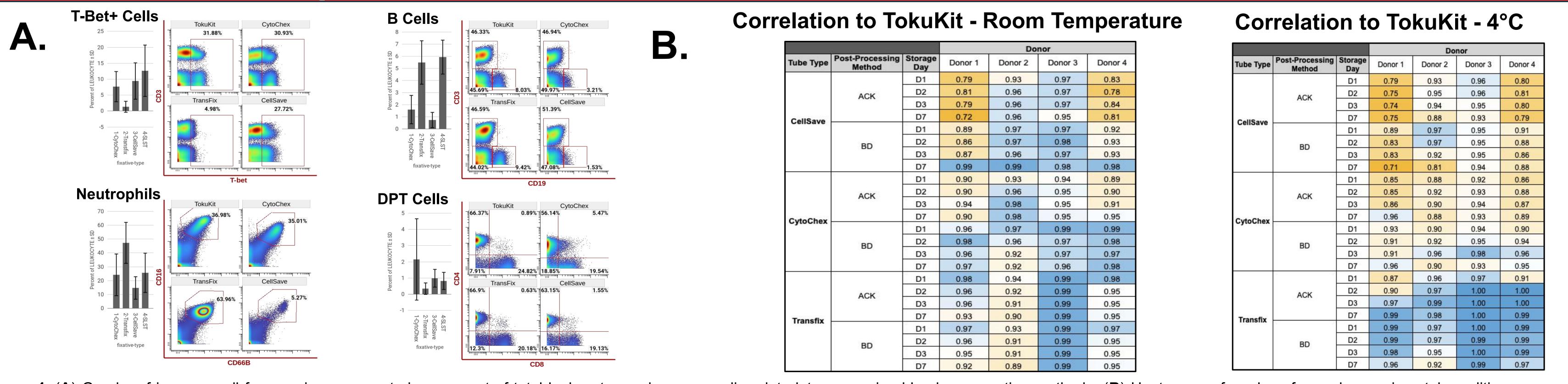


Figure 4. (**A**) Graphs of immune cell frequencies represented as percent of total leukocytes and corresponding dot plots comparing blood preservation methods. (**B**) Heat maps of r values for each experimental condition compared to TokuKit-Fixed whole blood at -80°C at 14 days.

Conclusions			
Cyto-Chex	Transfix	CellSave	TokuKit (-80°C storage)
Single tube collection	✓ Preserves cell population frequencies similar to TokuKit	Single tube collection	✓ Can store for long term (15+ months)
Preservation at ambient temperature	Struggle to separate monocyte subpopulations (CD14+/- and CD16	Preservation at ambient temperature	Highly correlated with fresh, live cell staining
up to 14 days	+/-) in gating	up to 4 days	Preserves immune cell frequencies
O Does not preserve B cell population frequencies on CyTOF.	Serial Failed to detect intracellular markers (e.g. T-bet)	O Does not preserve B cell population frequencies	
		Reduced total event count by CyTOF	