Comparison of Whole Blood Preservation Methods for Cytometric Analysis

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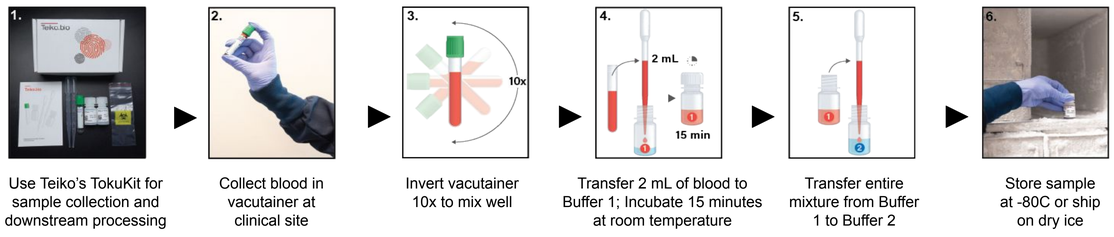
Introduction

Clinical trials rely on high-dimensional immune profiling to evaluate treatment responses, but inconsistencies in blood collection, shipping, and processing result in a 10–15% 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 such as T-bet. CellSave maintained overall immune frequencies and achieved the highest correlation to frozen controls at 7 days (r = 0.97 with BD Lyse) but exhibited slightly elevated granulocyte background.

TokuKit samples can be stored at –80°C for more than 6 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.

Teiko's TokuKit Workflow



Use Teiko's TokuKit for sample collection and downstream processing

Collect blood in vacutainer at clinical site

Invert vacutainer 10x to mix well

Transfer 2 mL of blood to Buffer 1; Incubate 15 minutes at room temperature

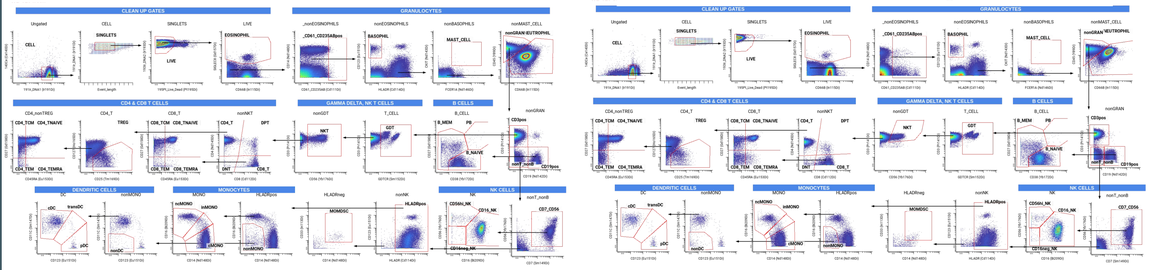
Transfer entire mixture from Buffer 1 to Buffer 2

Store sample at -80C or ship on dry ice

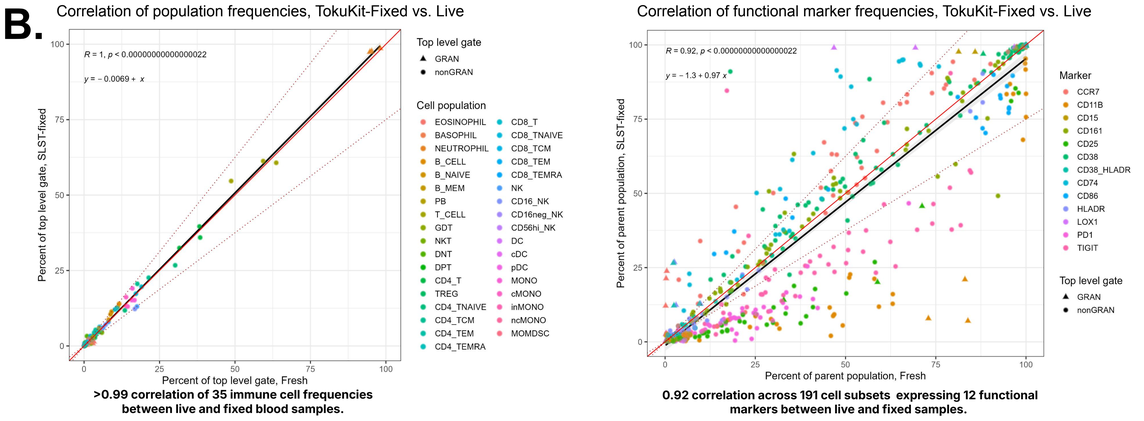
Figure 1. Schematic of the whole blood collection process using the TokuKit

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

A.



B.



>0.99 correlation of 35 immune cell frequencies between live and fixed blood samples.

0.92 correlation across 191 cell subsets expressing 12 functional markers between live and fixed samples.

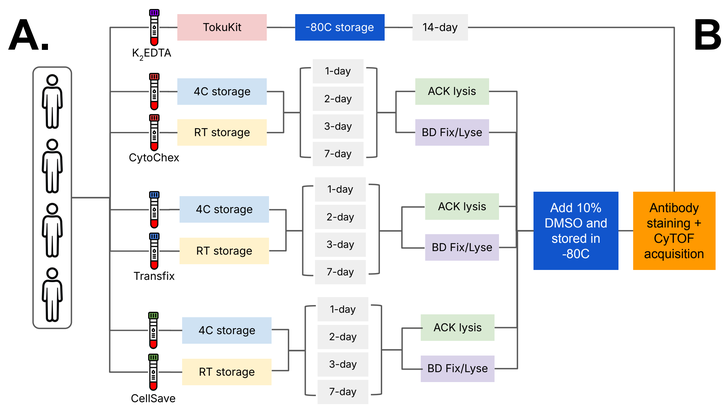
C.

Validation Parameter	Metric	Result	Acceptance Criteria
Intra-run precision	Average %CV across all measurable populations	**2.16%**	≥95% of measurements CV ≤ 25%
Inter-run precision	Average %CV across all measurable populations	**6.83%**	≥95% of measurements CV ≤ 30%
Post-collection stability (frozen at −80 °C)	Percent difference between t=0 and t=x month	**up to 15-month**	≤25% change between timepoints

Figure 2. (**A**) Schematic of gating strategy for Live (left) and TokuKit-Fixed (right) whole blood samples. (**B**) Correlation of population frequencies (left) and functional marker subset frequencies (right) between live and TokuKit-fixed samples stored for 14 days at -80°C. (**C**) Chart of intra- and inter-run precision across all measurable populations as well as long-term stability measures for TokuKit fixed whole blood samples.

Method and Panel for Comparison of Stabilization Methods

A.



B.

Metal	Mass	Marker	Clone
Y89	89	CD45	HI30
Cd	112	CD8	SK1
In	113	CD33	WM53
Cd	114	HLA-DR	L243
In	115	CD66b	G10F5
Cd	116	CD57	HNK-1
Pr	141	CD3	UCTH1
Dy	161	CTLA-4	14D3
Yb	171	CD15	W6D3
Bi	209	CD16	3G8
La	139	KLRG1	SA231A2
Nd	142	CD19	HIB19
Nd	143	CD69	FN50
Nd	144	Granzyme B	GB11
Nd	145	CD4	RPA-T4
Nd	146	CD11b	ICRF44
Sm	147	CD11c	Bu15
Nd	148	CD14	M5E2
Sm	149	TIGIT	MBSA43
Eu	15	CD123	6H6
Nd	150	CD86	IT2.2
Sm	152	gdTCR	B1
Eu	153	CD45RA	HI100
Sm	154	TIM3	F38-2E2
Gd	155	CD95	DX2
Gd	156	PD-L1	29E.2A3
Gd	157	CCR7	G043H7
Gd	158	CD27	L128
Tb	159	CD39	A1
Gd	160	Tbet	4B10
Dy	162	Foxp3	PCH101
Dy	163	CD28	L293
Dy	164	CD161	HP-3G10
Ho	165	CD127	R34-34
Er	166	CD74	LN2
Er	167	CD25	M-A251
Er	168	Ki67	Ki-67
Tm	169	ICOS	C398.4A
Er	170	LOX-1	15C4
Yb	172	CD38	HIT2
Yb	173	IgG4	HP6025
Yb	174	PD-1	EH12.2H7
Lu	175	LAG-3	11C3C65
Yb	176	CD56	NCAM16.2

Figure 3. (**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

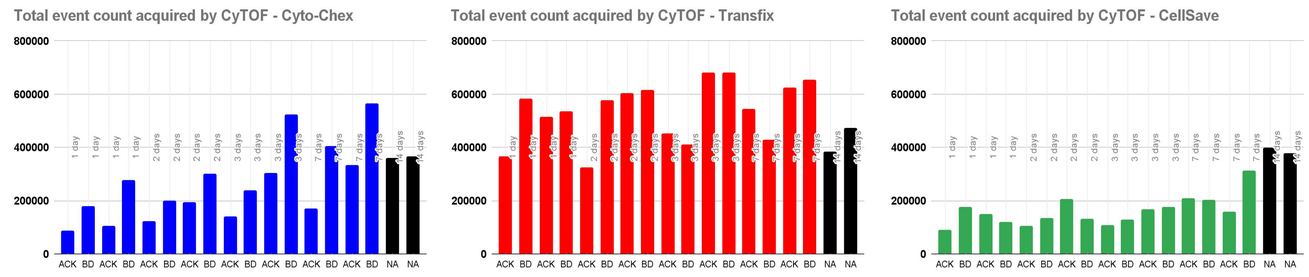
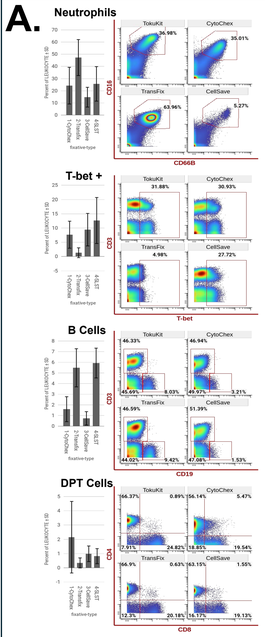


Figure 4. Comparison of total events acquired by CyTOF after blood collection in Cyto-Chex (blue), Transfix (red), and CellSave (green) compared to TokuKit (black).

Comparison of Whole Blood Preservation Methods to TokuKit

A.



B. **Correlation to TokuKit - Room Temperature**

Tube Type	Post-Processing Method	Storage Day	Donor 1	Donor 2	Donor 3	Donor 4
CellSave	ACK	D1	0.79	0.93	0.97	0.83
		D2	0.81	0.96	0.97	0.78
		D3	0.79	0.96	0.97	0.84
		D7	0.72	0.96	0.95	0.81
	BD	D1	0.89	0.97	0.97	0.92
		D2	0.86	0.97	0.98	0.93
		D3	0.87	0.96	0.97	0.93
		D7	0.99	0.99	0.98	0.98
CytoChex	ACK	D1	0.90	0.93	0.94	0.89
		D2	0.90	0.96	0.95	0.90
		D3	0.94	0.98	0.95	0.91
		D7	0.90	0.98	0.95	0.95
	BD	D1	0.96	0.97	0.99	0.99
		D2	0.98	0.96	0.97	0.98
		D3	0.96	0.92	0.97	0.97
		D7	0.97	0.92	0.96	0.98
Transfix	ACK	D1	0.98	0.94	0.99	0.98
		D2	0.96	0.92	0.99	0.95
		D3	0.96	0.91	0.99	0.95
		D7	0.93	0.90	0.99	0.95
	BD	D1	0.97	0.93	0.99	0.97
		D2	0.96	0.91	0.99	0.95
		D3	0.95	0.91	0.99	0.95
		D7	0.92	0.89	0.99	0.95

Correlation to TokuKit - 4°C

Tube Type	Post-Processing Method	Storage Day	Donor 1	Donor 2	Donor 3	Donor 4
CellSave	ACK	D1	0.79	0.93	0.96	0.80
		D2	0.75	0.95	0.96	0.81
		D3	0.74	0.94	0.95	0.80
		D7	0.75	0.88	0.93	0.79
	BD	D1	0.89	0.97	0.95	0.91
		D2	0.83	0.97	0.95	0.88
		D3	0.83	0.92	0.95	0.86
		D7	0.71	0.81	0.94	0.88
CytoChex	ACK	D1	0.85	0.88	0.92	0.86
		D2	0.85	0.92	0.93	0.88
		D3	0.86	0.90	0.94	0.87
		D7	0.96	0.88	0.93	0.89
	BD	D1	0.93	0.90	0.94	0.90
		D2	0.91	0.92	0.95	0.94
		D3	0.91	0.96	0.98	0.96
		D7	0.96	0.90	0.93	0.95
Transfix	ACK	D1	0.87	0.96	0.97	0.91
		D2	0.90	0.97	1.00	1.00
		D3	0.97	0.99	1.00	1.00
		D7	0.99	0.98	1.00	0.99
	BD	D1	0.99	0.97	1.00	0.99
		D2	0.99	0.97	0.99	0.99
		D3	0.98	0.95	1.00	0.99
		D7	0.96	0.92	0.99	0.97

Figure 5. (**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.

Conclusions

CytoChex	Transfix	CellSave	TokuKit (-80C storage)
✅ Single tube collection	✅ Preserves cell population frequencies similar to TokuKit	✅ Single tube collection	✅ Can store for long term (6+ months)
✅ Preservation at ambient temperature up to 14 days	🚫 Struggle to separate monocyte subpopulations (CD14+/- and CD16 +/-) in gating	✅ Preservation at ambient temperature up to 4 days	✅ Highly correlated with fresh, live cell staining
🚫 Does not preserve B cell population frequencies	🚫 Failed to detect intracellular markers (e.g. T-bet)	🚫 Does not preserve B cell population frequencies	✅ Preserves immune cell frequencies
🚫 Increase double positive T (CD4+CD8+) that may be artifacts when stored for >7 days		🚫 Reduced total event count by CyTOF	

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