

# You're in the right place.

To follow along at home, download our report at  
**[teiko.bio/spectral-flow-cytometry/#cia-validation-data](https://teiko.bio/spectral-flow-cytometry/#cia-validation-data)**

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Teiko.bio

# **CLIA Validation** for our **25-marker spectral flow test**

## **High-parameter cytometry for clinical trials**



**Ramji Srinivasan**  
**Teiko CEO**

# What we tested

[teiko.bio/spectral-flow-cytometry/#clia-validation-data](https://teiko.bio/spectral-flow-cytometry/#clia-validation-data)

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# Spectral: Immune Profiling Markers

## Our panel includes:

- All major immune cell population and subsets
- 5 functional state markers across subsets

## T cells

CD3	Total T cells
CD4	CD4+ T cells
CD8	CD8+ T cells
CD25	Treg, activation
CD127	T cell subsets
CD45RA	Naive/Memory
CD27	Maturation
CD28	Maturation
TCRgd	gdT cells
CD56	NKT cells
CD38	Activation
HLA-DR	Activation
PD-1	Exhaustion

## B cells

CD19	Total B cells
CD20	B cell subsets
CD27	Naive/Memory
IgD	B cell subsets
IgM	B cell subsets
CD25	Activation
HLA-DR	Antigen presentation

## NK cells

CD56	NK cell subsets
CD16	NK cell subsets
CD38	Activation

## Myeloid cells

CD14	Monocyte subsets
CD16	Monocyte subsets
CD11c	Monocytes, macrophages, DCs
CD123	pDC
CD141	cDC1
CD1C	cDC2
HLA-DR	Antigen presentation

## Granulocytes

CD123	Basophil
CD66b	Granulocytes

## General

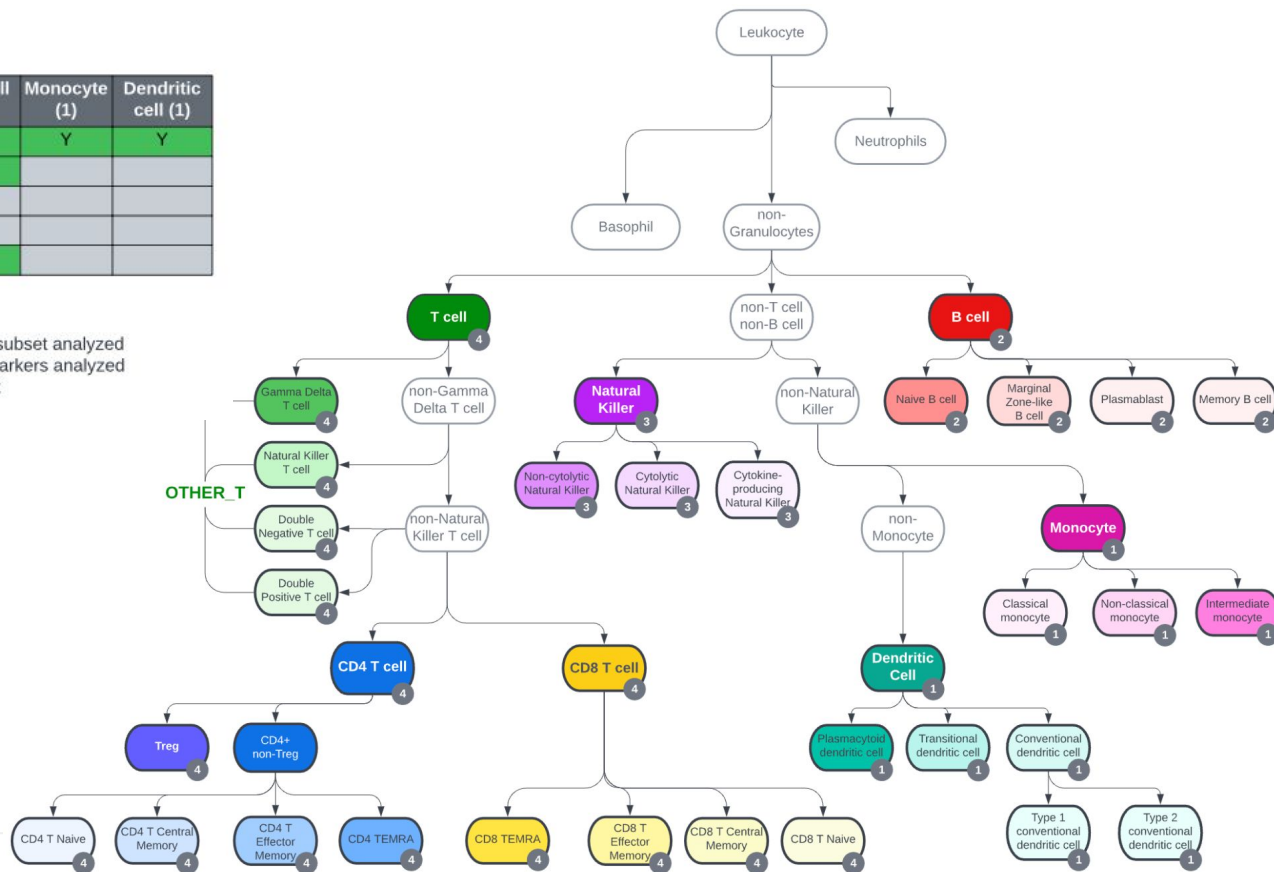
CD45	Total immune cells
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# Spectral Whole Blood: Cell populations and state markers

State Markers (5)	T cell (4)	B cell (2)	NK cell (3)	Monocyte (1)	Dendritic cell (1)
PD-1	Y	Y	Y	Y	Y
HLA-DR	Y		Y		
CD38	Y				
CD25	Y	Y			
CD8			Y		

## Legend

**X cell** <sub>N</sub> X cell: Name of immune subset analyzed  
 N: Number of cell state markers analyzed within this immune subset



# Tests used TokuKit fixed whole blood (WB) from three healthy human donors

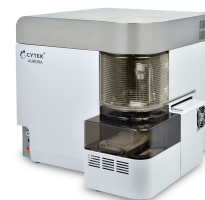
Acquired samples from three healthy donors

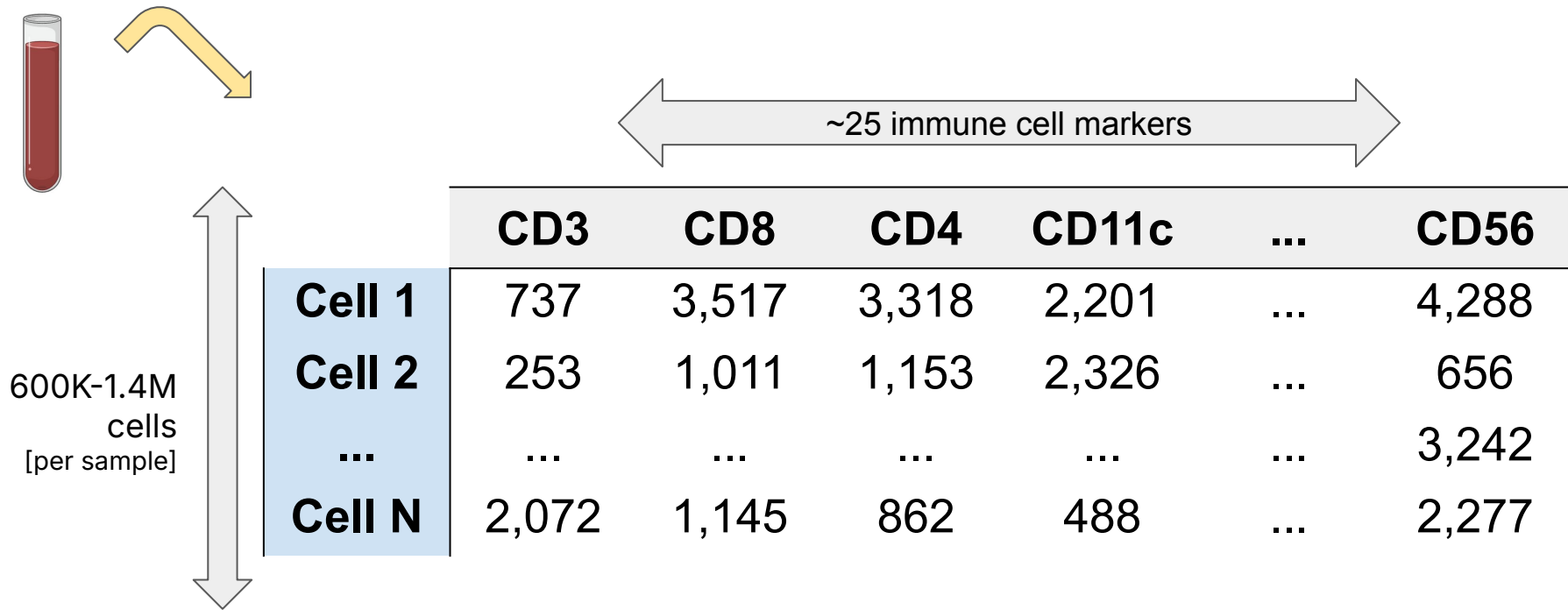


TokuKit fixation at Teiko



Samples evaluated in test





Each entry is a signal intensity

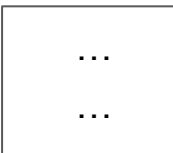
# How do you reliably measure the immune state?

Measurement 1



25

200,000

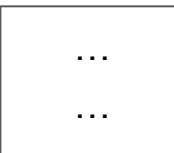


Measurement 2



25

200,000

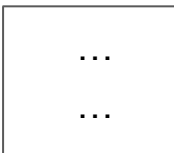


Measurement 3



25

200,000





**Is this test  
reproducible?**

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# Validation Plan

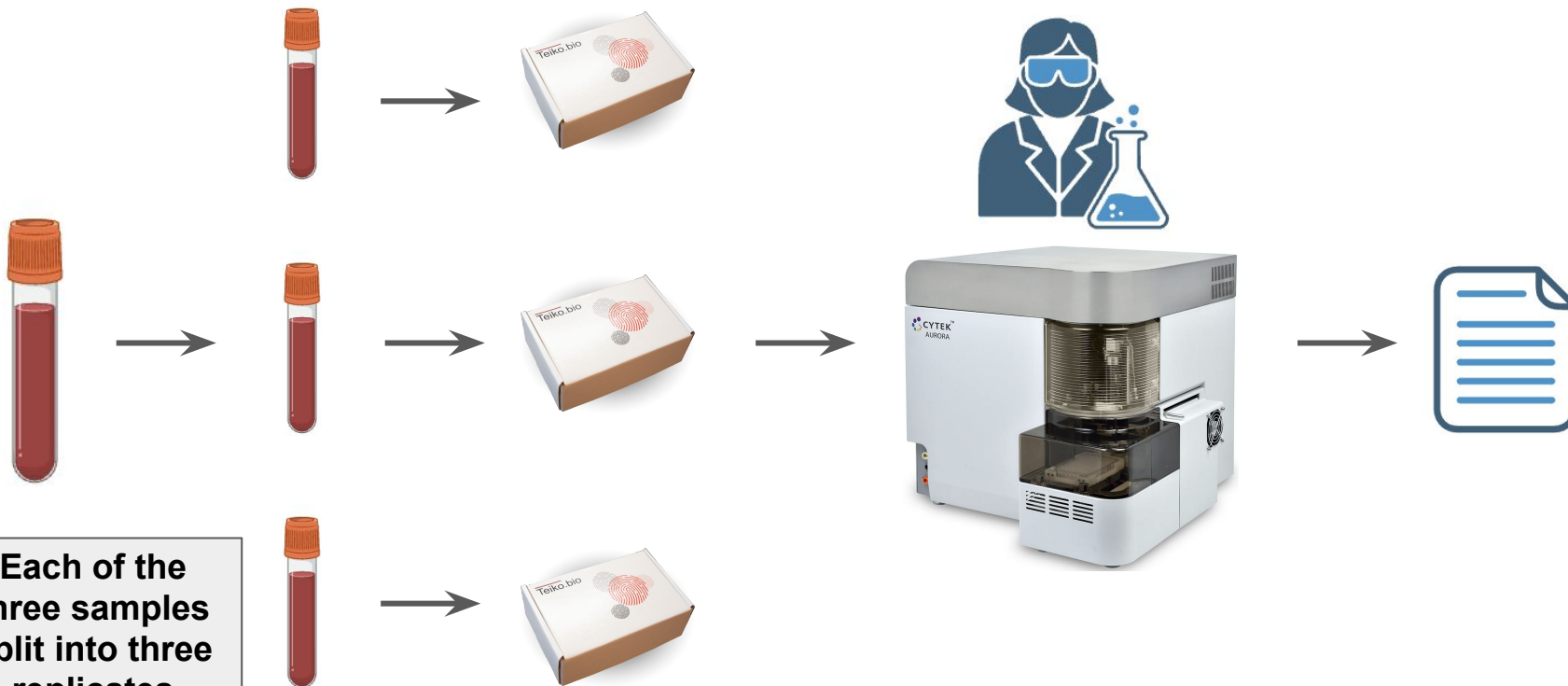
Measure	Meaning	Acceptance Criteria	Immune Populations Analyzed Per Donor*	Total Average Criteria Readout (%)	Total Pop. Below Criteria (%)
Intra-Run Precision	Same sample, same run	CV $\leq$ 20% for $\geq$ 95% of pop.	32	To come	
Inter-Run Precision	Same sample, different runs	CV $\leq$ 20% for $\geq$ 95% of pop.	32		

\*Only populations (pop.) with >100 median cells across three donors were included in analysis

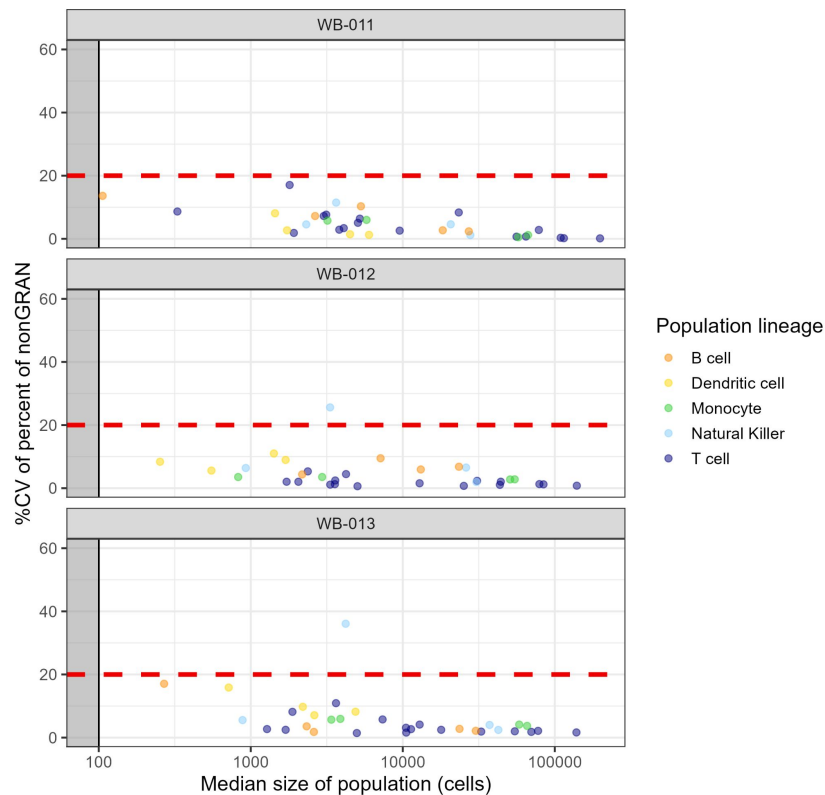
# Intra-run

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# Intra-Run: Same sample, same run



# Intra-Run by cell population size



**Average %CV across all  
three donors:  
4.76**

# Validation Results

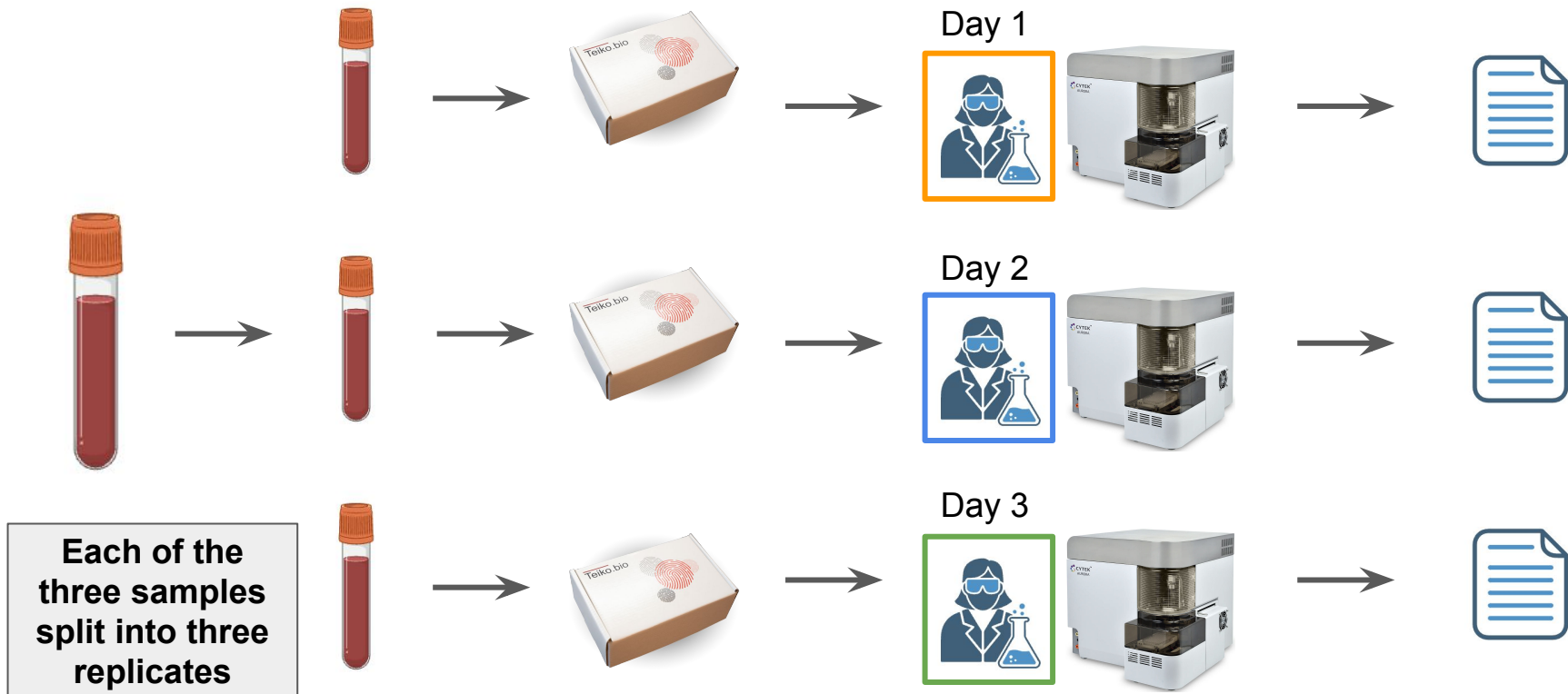
Measure	Meaning	Acceptance Criteria	Immune Populations Analyzed Per Donor*	Total Average Criteria Readout (%)	Total Pop. Below Criteria (%)
Intra-Run Precision	Same sample, same run	CV $\leq$ 20% for $\geq$ 95% of pop.	32	4.76 %	100 % (32/32)
Inter-Run Precision	Same sample, different runs	CV $\leq$ 20% for $\geq$ 95% of pop.	32		To come

\*Only populations (pop.) with >100 median cells across three donors were included in analysis

# Inter-run

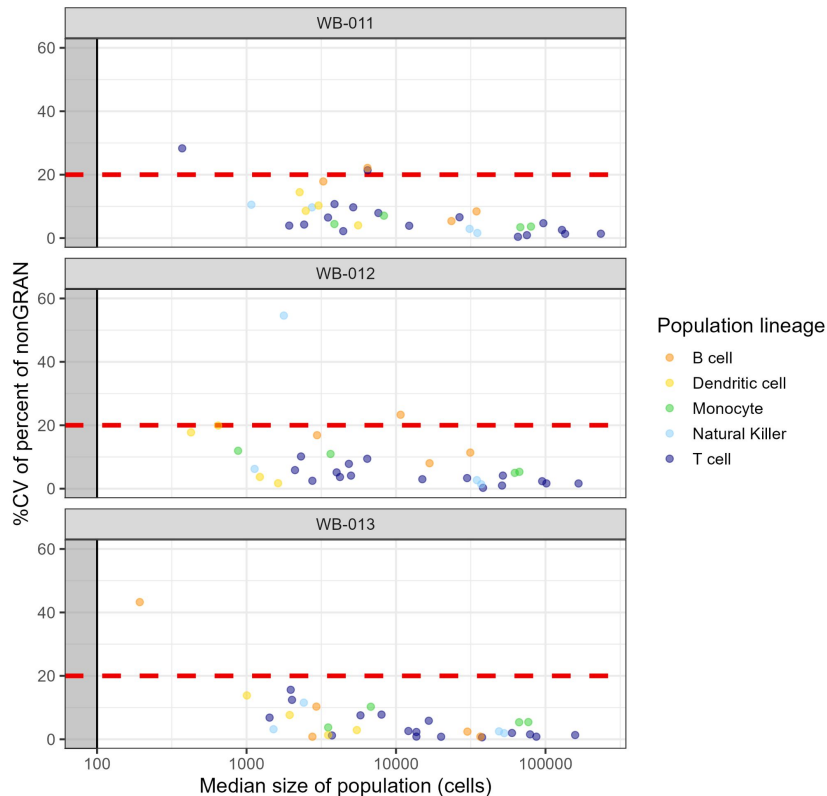
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# Inter-Run: Same sample, different days, different operators





# Inter-Run Precision by cell population size



**Average %CV across all three donors:**  
**6.89**

# Validation Results

Measure	Meaning	Acceptance Criteria	Immune Populations Analyzed Per Donor*	Total Average Criteria Readout (%)	Total Pop. Below Criteria (%)
Intra-Run Precision	Same sample, same run	CV $\leq$ 20% for $\geq$ 95% of pop.	32	4.76 %	100 % (32/32)
Inter-Run Precision	Same sample, different runs	CV $\leq$ 20% for $\geq$ 95% of pop.	32	6.89 %	96.8 % (31/32)

\*Only populations (pop.) with >100 median cells across three donors were included in analysis

**Is this test  
comparable to fresh  
blood?**

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# Check out our previous webinar:



Overall correlation of population frequencies between live and fixed sample processing: **0.97**

# Why fix blood instead of running fresh?

## 1. **10-20X reduction of failure rate through centralization of flow analysis**

No need for a flow lab in every country, city, clinical site. Reduce variability by having all samples processed by the same facility.

## 2. **Enables batching of samples**

Conveniently store fixed samples until a whole set (for example all timepoints of an individual patient) is complete.

## 3. **Reduce committed processing cost and expand analysis window**

Not sure which samples to analyze? No problem. Store samples at -80C until you're ready to decide which samples to analyze.

# Read on

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# Appendix

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# Complete Gating Strategy

