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You're in the right place.

To follow along at home, download our report at
teiko.bio/technology/#clia-validation-data

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Read along at home

Method Validation Plan

A Mass Cytometry Assay to Determine the Pan-Immune Profile of PBMCs Isolated from Healthy Human Clinical Samples

Method Validation Report

A Mass Cytometry Assay to Determine the Pan-Immune Profile of PBMCs Isolated from Healthy Human Clinical Samples

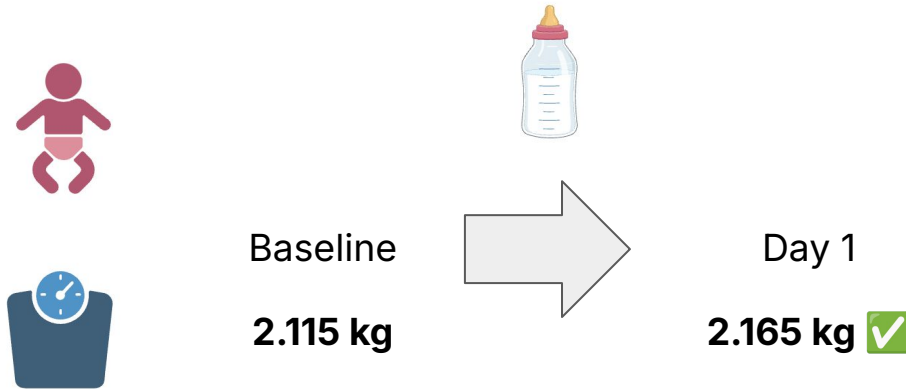July 2023
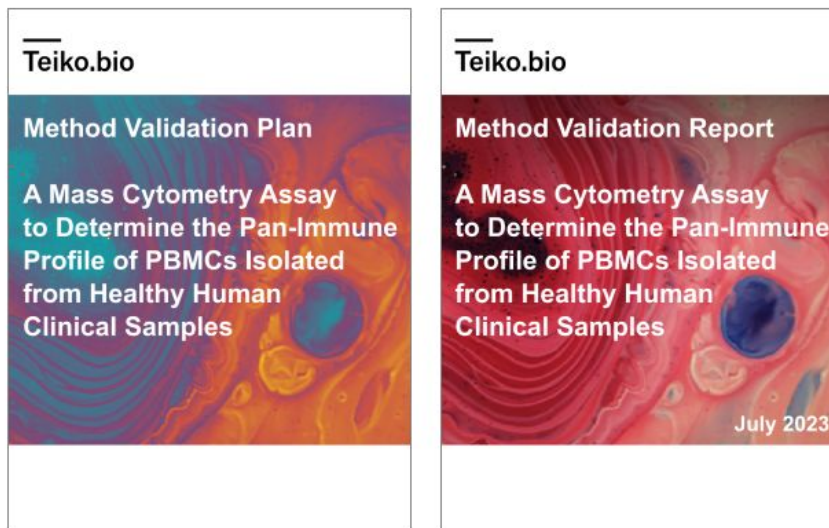
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Problem

High-stakes reliable measurement

How do you reliably weigh a crying baby at low birth weight?

Low birth weight babies that don't gain weight quickly need medical intervention

Baseline

2.115 kg

Day 1

2.165 kg ✅

Need a weight scale that can reliably detect weight gain

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How do you reliably weigh a crying baby?

Measurement 1	Measurement 2	Measurement 3
2.165 kg	**2.160 kg**	**2.155 kg**

Baseline: 2.115 kg

Now think about the immune state

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How do you reliably measure the immune state?

Baseline → Day 1

Baseline
T cells
B cells
NK cells
Monocytes
Dendritic Cells

Day 1
T cells
B cells
NK cells
Monocytes
Dendritic Cells
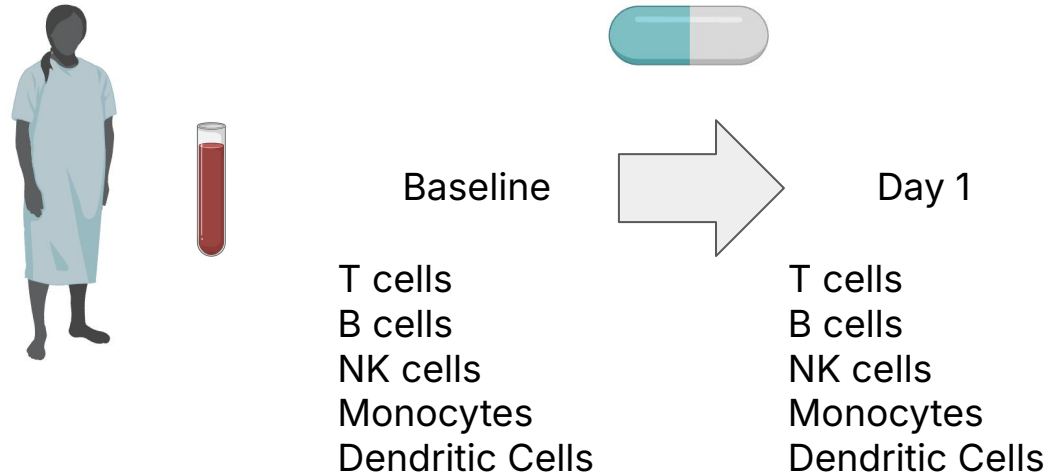
Need immune "scale" that can reliably measure immune response across cells

How do you reliably measure the immune state?

Measurement 1

Markers

Cells   40

200,000   …

              …

Measurement 2

Markers

Cells   40

200,000   …

              …

Measurement 3

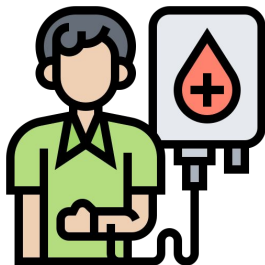Markers

Cells   40

200,000   …
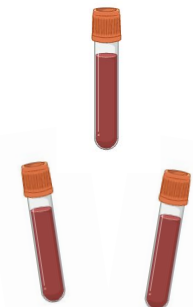
              …

What we tested

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Tests used Peripheral Blood Mononuclear Cells (PBMCs) isolated in-house from healthy donors
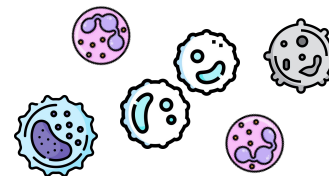
Healthy donors (n=9)

Blood sent to Teiko

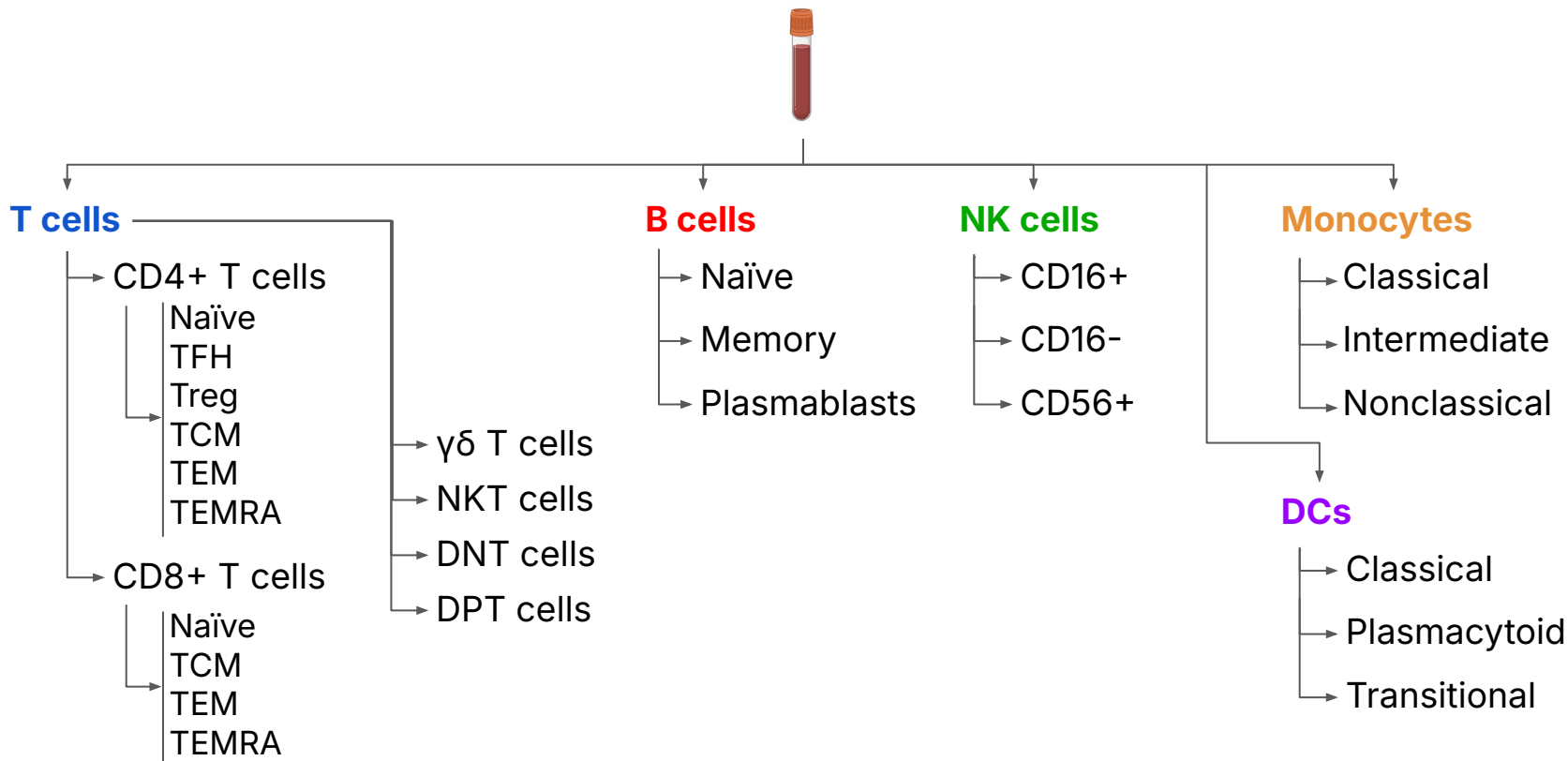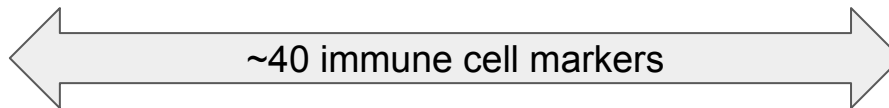**PBMC Isolation**

PBMCs used in tests

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Teiko's Pan-Immune Profiling PBMC Test

T cells
- → CD4+ T cells
  - Naïve
  - TFH
  - Treg
  - TCM
  - TEM
  - TEMRA
- → CD8+ T cells
  - Naïve
  - TCM
  - TEM
  - TEMRA

- → γδ T cells
- → NKT cells
- → DNT cells
- → DPT cells

B cells
- → Naïve
- → Memory
- → Plasmablasts

NK cells
- → CD16+
- → CD16-
- → CD56+

Monocytes
- → Classical
- → Intermediate
- → Nonclassical

DCs
- → Classical
- → Plasmacytoid
- → Transitional

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~40 immune cell markers

	CD3	CD8	CD4	CD11c	...	Tbet
Cell 1	737	3,517	3,318	2,201	...	4,288
Cell 2	253	1,011	1,153	2,326	...	656
...	...	...	...	...	...	3,242
Cell N	2,072	1,145	862	488	...	2,277

200-400K cells [per sample]

Each entry is a signal intensity

How do you reliably measure the immune state?

Measurement 1

Measurement 2

Measurement 3

200,000

40

…

…

200,000

40

…

…

200,000

40

…

…

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Is this thing valid?

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Validation Plan
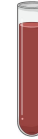
Measure	Meaning	Acceptance Criteria	Total Immune Subsets Analyzed*	Total Average Coefficient of Variation (%)	Total Average Change (%)
Intra-Run Precision	Same sample, same run	CV ≤ 25% between replicates			
Inter-Run Precision	Same sample, different runs	CV ≤ 30% between runs	We'll go through these numbers in the next few slides		
Inter-Operator Precision	Two donors, fixed by different operators	CV ≤ 25% between operators			
Stability	Same sample, across time	Change ≤ ±25% between days			

*Only populations with >100 median cells were included in analysis

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Coefficient of Variation

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True Weight [kgs]	2.165
Baseline Weight [kgs]	2.115

Measurement	Good Scale Measured weight	Bad Scale Measured weight
1	2.16	3.66
2	2.17	3.34
3	2.21	1.48
4	2.22	1.30
5	2.24	1.96
Coefficient of variation	1.44%	46.20%
Standard Deviation	0.0317	1.0851
Mean	2.1997	2.3489

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Intra-run

Intra-Run Precision: Same sample, same run

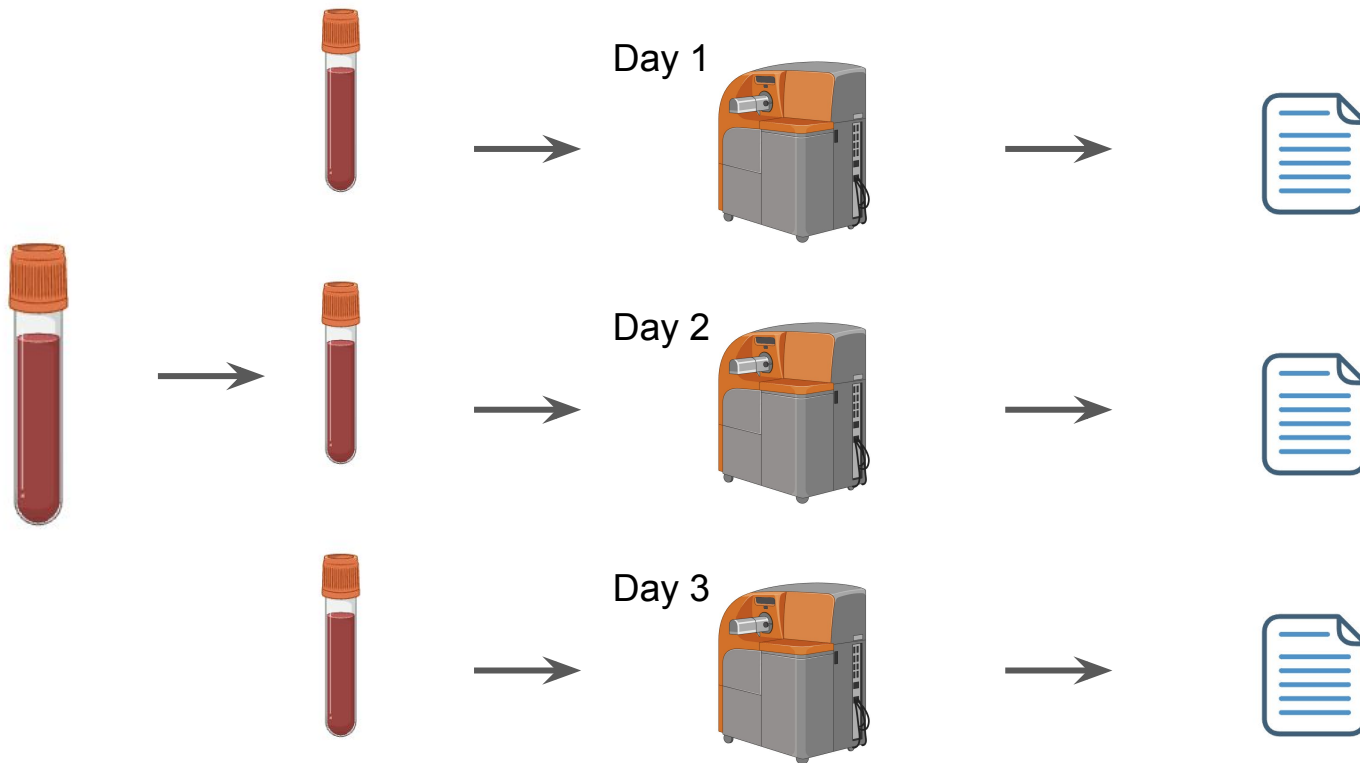Teiko.bio
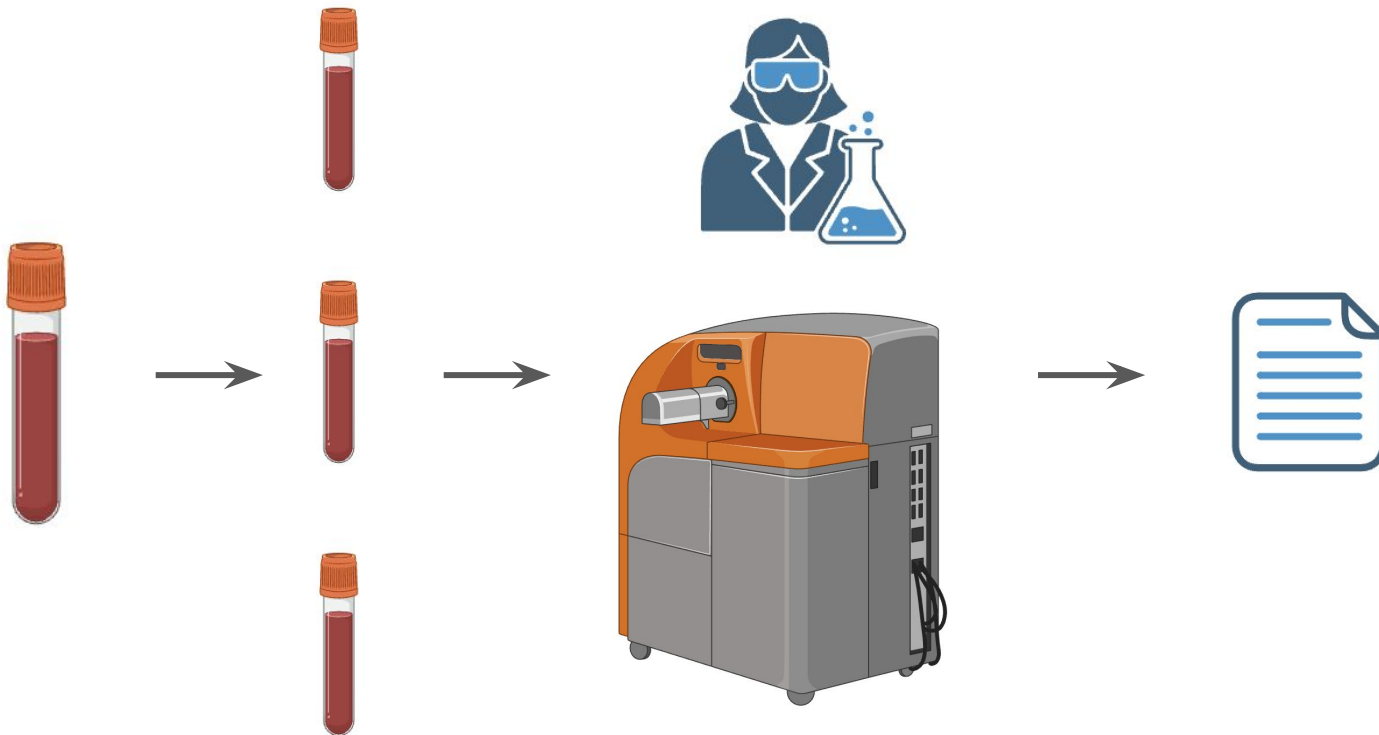
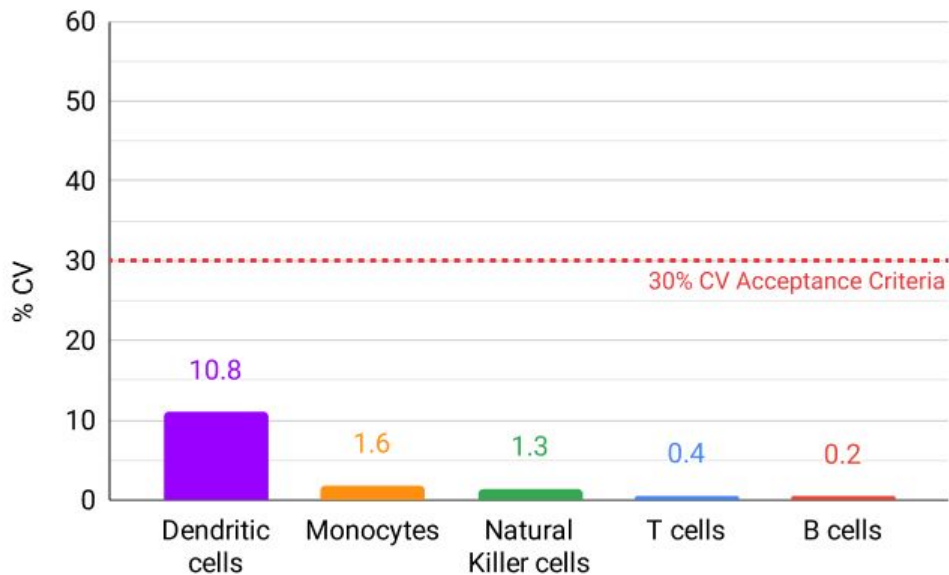# Intra-Run Precision by major lineage

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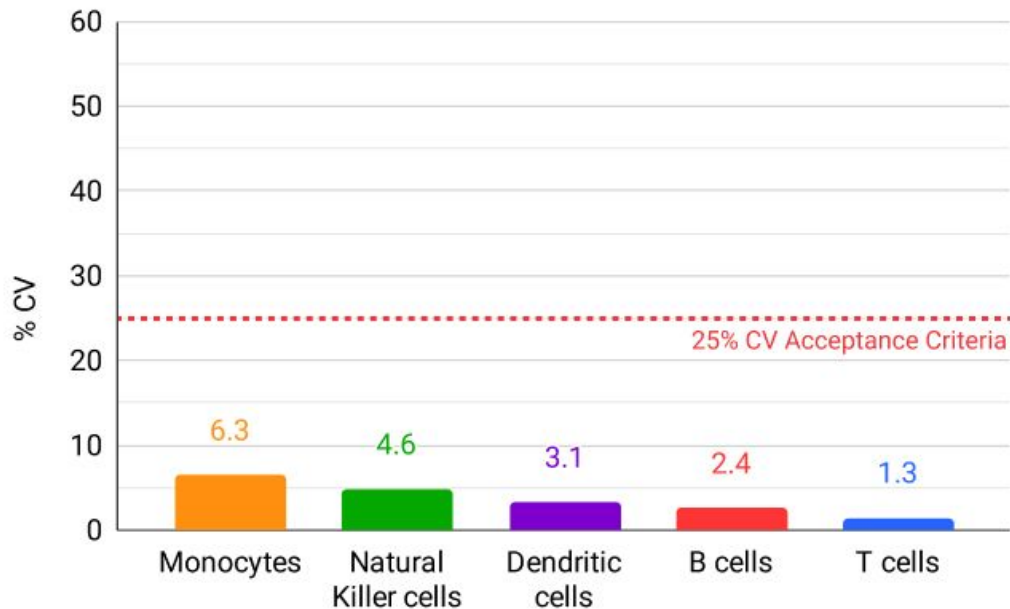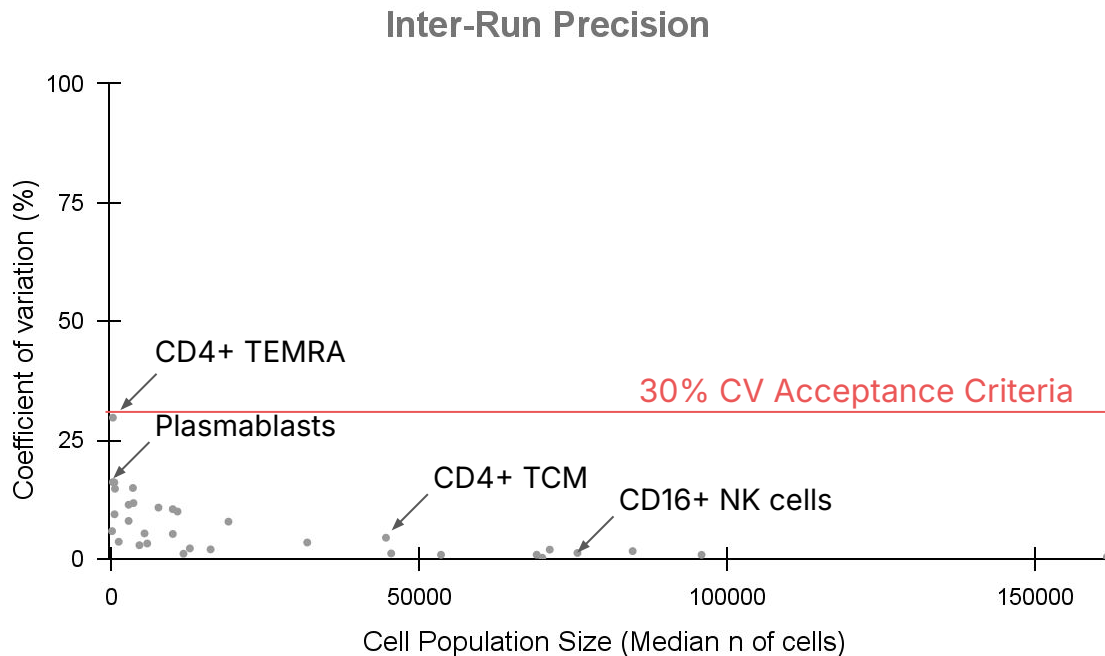
Inter-run

Inter-Run Precision: Same sample, different days

Day 1

Day 2

Day 3

Inter-Run Precision by major lineage

Average %CV: 6.66

Inter-Run Precision by cell population size

Inter-Run Precision

A cell population with fewer cells is more variable
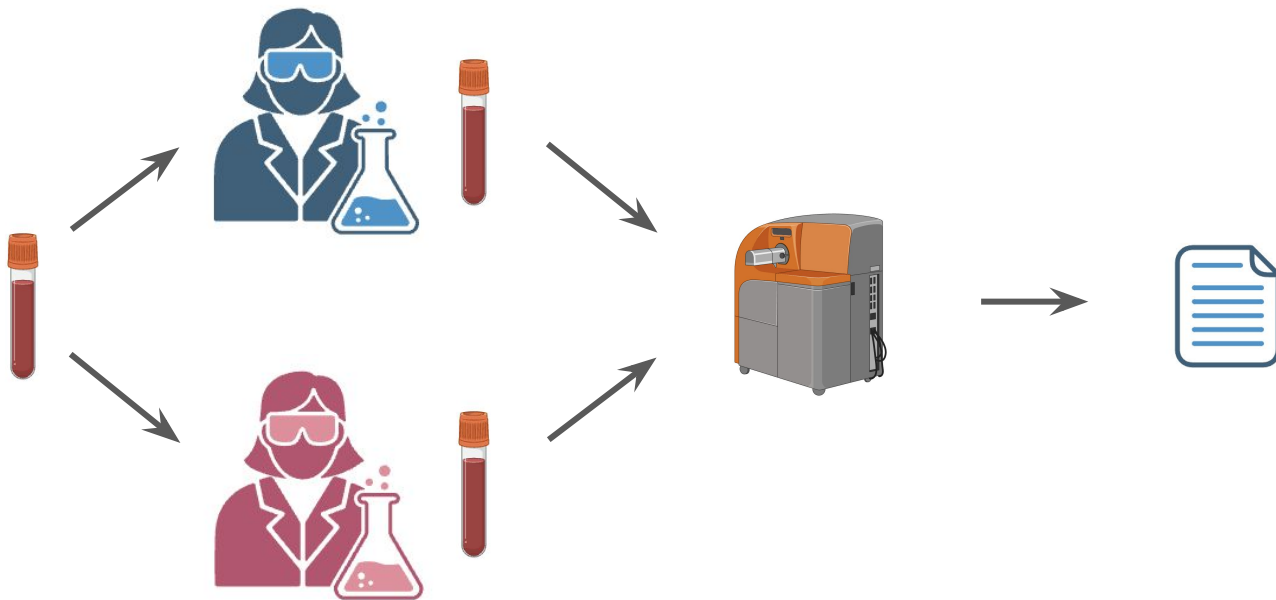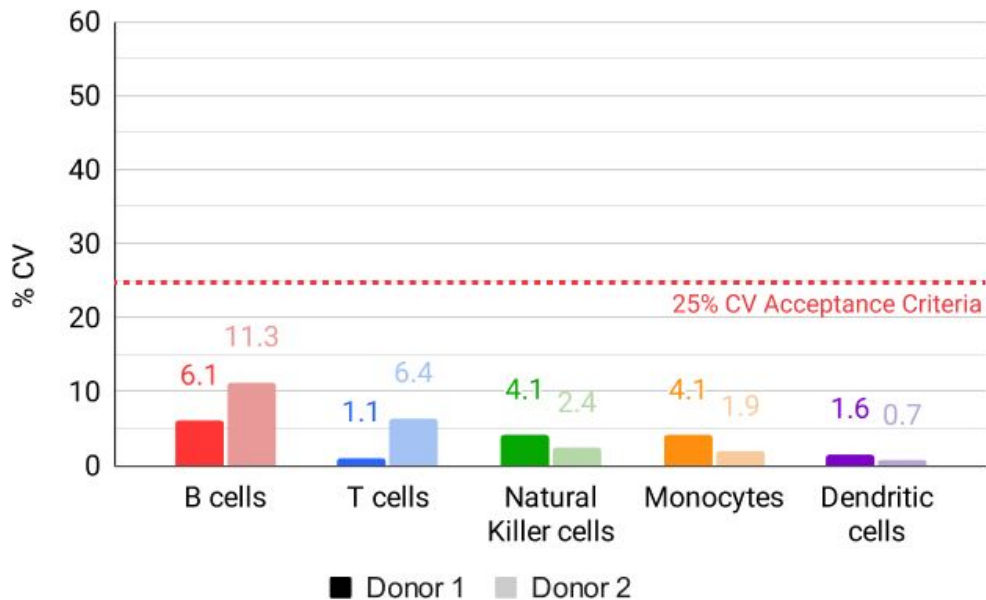
Inter-operator

Inter-Operator Precision: Two donors, fixed by different operators

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Inter-Operator Precision by major lineage

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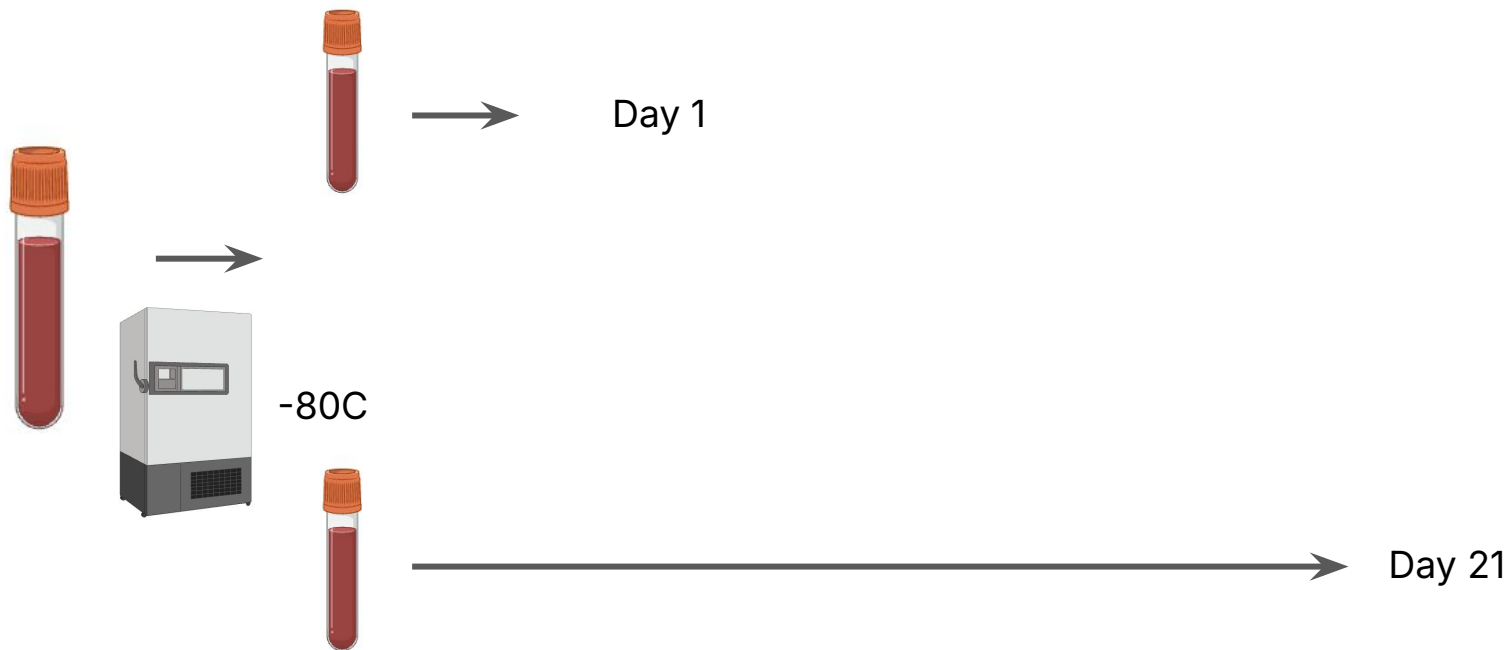

Average %CV:
Donor 1: 4.83
Donor 2: 7.36

Stability

Stability: same sample, processed after 1 or 21 days

Day 1

-80C

Day 21

Stability by major lineage
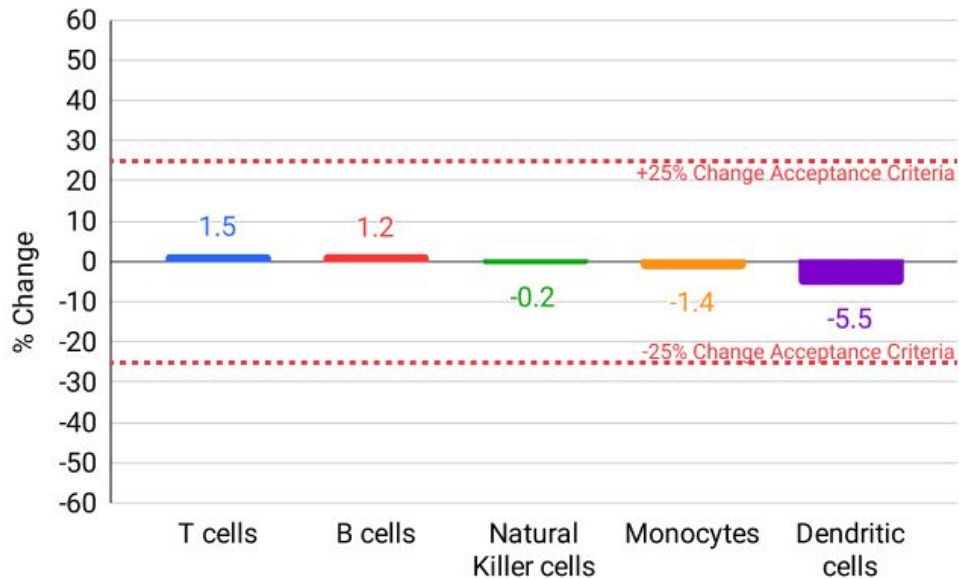
Average %Change:
-0.01

Is this thing valid?

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Validation Plan

Measure	Meaning	Acceptance Criteria	Total Immune Subsets Analyzed*	Total Average Coefficient of Variation (%)	Total Average Change (%)
Intra-Run Precision	Same sample, same run	CV ≤ 25% between replicates			
Inter-Run Precision	Same sample, different runs	CV ≤ 30% between runs		Here we go!	
Inter-Operator Precision	Two donors, fixed by different operators	CV ≤ 25% between operators			
Stability	Same sample, across time	Change ≤ ±25% between days			

*Only populations with >100 median cells were included in analysis

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Yes!

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Measure	Meaning	Acceptance Criteria	Total Immune Subsets Analyzed*	Total Average Coefficient of Variation (%)	Total Average Change (%)
Intra-Run Precision	Same sample, same run	CV ≤ 25% between replicates	30	3.52%	--
Inter-Run Precision	Same sample, different runs	CV ≤ 30% between runs	33	6.66%	--
Inter-Operator Precision	Two donors, fixed by different operators	CV ≤ 25% between operators	30	• Donor 1: 4.83% • Donor 2: 7.36%	--
Stability	Same sample, across time	Change ≤ ±25% between days	29	--	-0.01%

*Only populations with >100 median cells were included in analysis

Well suited for demanding clinical trials
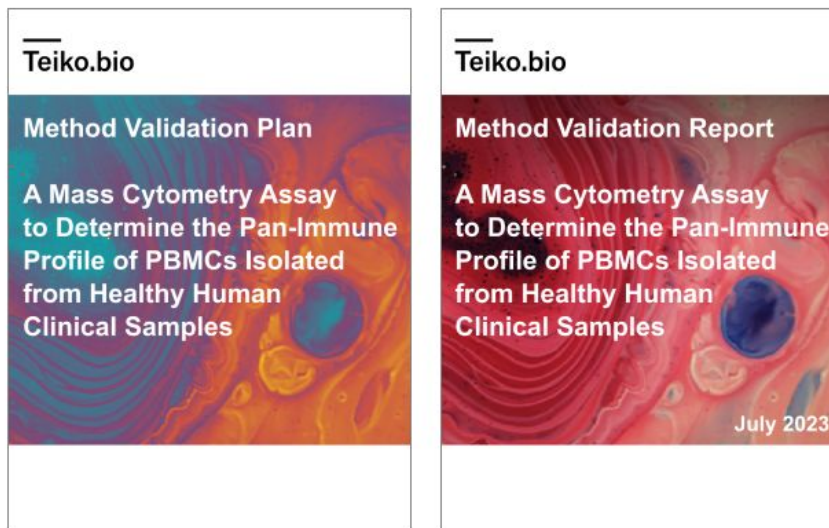
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Thank you!

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To measure the immune state

- Presence or absence of dozens of different individual cell types across hundreds of thousands of cells
- Presence or absence of unique markers on those cells
- Proportion of those cells in relation to one another

Teiko's Pan-Immune Profiling PBMC Panel (1/2)

Carefully selected antigens identify:
○ All major immune subpopulations
○ Important activation and / or maturation proteins

T cells

T cells

CD3	Total T cells
CD4	CD4+ T cells
CD8a	CD8+ T cells
CD25	Treg, activation
CD27	Maturation
CD28	Costimulation
CD38	Maturation

CD39	Activation
CD45RA	Naive/memory cells
CD56	NKT cells, T cell subsets
CD57	Senescence
CD69	Activation
CD127	T cell subsets
CD152 (CTLA4)	Checkpoint
CD161	T cell subsets
CD197 (CCR7)	Naive/memory cells
CD279 (PD-1)	Checkpoint
Foxp3	Treg cells
gdTCR	gd T cells
Granzyme B	Cytotoxicity
HLA-DR	Activation
ICOS	Checkpoint
KLRG1	Inhibition
LAG-3	Checkpoint
T-bet	T cell subsets
TIGIT	Checkpoint
Tim-3	Checkpoint

Teiko's Pan-Immune Profiling PBMC Panel (2/2)

Antigen-presenting cells and NK cells

B cells

CD19	Total B cells
CD25	Activation
CD27	Naive/memory
CD38	Naive/memory
CD74	Antigen presentation
CD86	Costimulation
HLA-DR	Antigen presentation
IgG4	B cell subsets

Myeloid cells

CD11b	Monocytes, macrophages
CD11c	Monocytes, macrophages, DCs
CD14	Monocyte subsets, macrophages
CD15	Myeloid subsets
CD16	Monocyte subsets
CD33	Total myeloid

NK cells

CD8a	NK cell subsets
CD16	NK cell subsets
CD38	Activation
CD56	NK cell subsets
CD57	Maturation
CD69	Activation
CD161	NK cell subsets
Granzyme B	Cytotoxicity
KLRG1	Inhibition
T-bet	Maturation
TIGIT	Checkpoint

CD86	Costimulation
CD123	pDCs
LOX-1	Migration
PD-L1	Checkpoint ligand
HLA-DR	Antigen presentation

General

CD45
CD66b
Ki67
DNA
live/dead