



Method Validation Report for A Mass Cytometry
Assay to Determine the Pan-Immune Profile of
PBMCs Isolated from Human Whole Blood

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	Title: Performance Validation Report (Pan-Immune Profiling Test in PBMCs)
	Document Number: 122 Revision: 02
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2. Compliance Statement

The validation experiments were performed as per the validation study plan described in DCS086 CyTOF PBMC Performance Validation Plan, which is based on guidance from DCS034 Quality Assurance and Performance Verification. Any deviation to this validation or any relevant SOP will be described in detail and documented as an amendment to this validation report. The validation will be performed as per the Clinical Laboratory Improvement Amendments (CLIA) guidelines.

3. Purpose

This document describes the performance validation results for Teiko Bio's Pan-Immune Profiling test. This validation report was created and reviewed before any patient testing occurred. Teiko Bio's Pan-Immune Profiling test is a laboratory-developed test that evaluates major human immune populations from peripheral blood mononuclear cells (PBMCs), including subsets in T cells, B cells, NK cells, monocytes and dendritic cells using mass cytometry (CyTOF). The validation was designed to evaluate a newly developed method.

4. Scope

The scope of this Fit-for-Purpose validation study is to demonstrate the robustness of the laboratory developed Pan-Immune Profiling Test, based on mass cytometry, to measure immune subsets from PBMCs isolated from whole blood collected from healthy human clinical specimens. The method follows the general development and validation procedure described in DCS086 Performance Validation Plan and following the general guidance described in DCS034 Quality Assurance and Performance Verification. This validation report is not intended to, nor does it, meet the regulatory requirements of the FDA for approval to market an in vitro diagnostic device (i.e., 510(k), PMA). This validation report also describes method development procedures that impact accuracy, which were established before validating the assay. In addition to method development procedures, this document describes the following performance validation results:

1. Accuracy
2. Precision
3. Stability
4. Reference Ranges

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The validation parameters were assessed in PBMCs isolated from healthy human donors. The test results in this document apply to the Pan-Immune Profiling Test provided by Teiko Bio located at 675 S Arapen Dr. Suite 301, Salt Lake City, Utah 84108.

5. Responsibility

The Laboratory Director of the laboratory and the General Supervisor are responsible for ensuring that all current testing personnel have reviewed and acknowledged the document prior to working on clinical samples.

This document will be reviewed annually unless otherwise deemed necessary.

In case any portion of this validation is revised, revisions will be performed and an amendment will be prepared for approval, documenting any aspects of the validation that were changed or updated from the previous version. Upon approval, the amendment will be included as part of the validation documents.

6. Acronyms

Acronym	Definition
PBMC	Peripheral blood mononuclear cells
CV	Coefficient of variation
TFH	T follicular helper cells
Treg	Regulatory T cells
TCM	Central Memory T cells
TEM	Effector Memory T cells
TEMRA	CD45RA+ Effector Memory T cells
NKT	Natural Killer T cells
NK cells	Natural Killer cells
DNT	CD4/CD8 Double-Negative T cells
DPT	CD4/CD8 Double-Positive T cells

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PB	Plasmablasts
cDC	Classical dendritic cells
pDC	Plasmacytoid dendritic cells
tDC	Transitional dendritic cells
cMono	Classical monocytes
inMono	Intermediate monocytes
ncMono	Non-classical monocytes
EDTA	Ethylenediaminetetraacetic acid
PBS	Phosphate-buffered saline
RT	Room temperature
PEB	Protein extraction buffer
PFA	Paraformaldehyde
CSB	Cell staining buffer
BSA	Bovine serum albumin
PMA	Phorbol 12-myristate 13-acetate
PHA	Phytohemagglutinin
FCS	Flow cytometry standard
SOP	Standard operating procedures

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7. Definitions

Term	Definition
Performance validation	Any systematic process of checking equipment against predefined process requirements, ensuring continuous operation within specifications and compliance with quality standards
Accuracy	Verification that the assay is able to measure what it proposes to measure
Precision	Closeness of agreement between independent test results obtained under stipulated conditions; reproducibility
Limit of detection	The lowest amount of analyte in a sample that can be consistently and reliably detected
Stability	Closeness of agreement between independent test results obtained at different points in time
Gating	The process of selecting and separating cell populations of interest from a heterogeneous mixture of cells, based on specific markers or properties.

8. Content

8.1 Objective

To validate the performance of Teiko Bio's Pan-Immune Profiling test for the analysis of major immune subpopulations for T cells, B cells, NK cells and myeloid cells in peripheral blood mononuclear cells (PBMCs) isolated by Ficoll Density Gradient at Teiko Bio.

8.2 Assay Design

Teiko Bio's Pan-Immune Profiling test panel is a mass cytometry-based test that has been designed to investigate the frequencies of major human immune subpopulations for T cells, B cells, NK cells, and myeloid cells. 44 antibodies are used to stain PBMCs collected by Ficoll Density Gradient from whole blood isolated from healthy human donors. In this report, the performance of the panel is described for different validation parameters: accuracy, precision,

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and stability. We also set a reference range for healthy subjects. See Table 1 for definitions for individual cell subsets detected in this assay.

Table 1: Teiko Bio's Pan-Immune Profiling Test cell subset definitions

Non-granulocytes are defined as CD45+ CD66b- Live Singlets. All subsets are measured as % of total non-granulocytes.

Cell Subset	Definition (Marker Parameters)
T cells	CD3+ CD19- CD33- CD14-
CD4+ T cells	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4+ CD8-
Naive	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4+ CD8- CD25- CD27+ CD45RA+
Follicular Helper (TFH)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4+ CD8- CD25- PD-1+ ICOS+
Regulatory T cells (Treg)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4+ CD8- CD127- CD25+ FOXP3+
Central Memory (TCM)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4+ CD8- CD25- CD27+ CD45RA-
Effector Memory (TEM)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4+ CD8- CD25- CD27- CD45RA-
CD45RA+ Effector Memory (TEMRA)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4+ CD8- CD25- CD27- CD45RA+
CD8+ T cells	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4- CD8+
Naive	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4- CD8+ CD27+ CD45RA+
Central Memory (TCM)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4- CD8+ CD27+ CD45RA-
Effector Memory (TEM)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4- CD8+ CD27- CD45RA-
CD45RA+ Effector Memory (TEMRA)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4- CD8+ CD27- CD45RA+
Gamma Delta ($\gamma\delta$) T cells	CD3+ CD19- CD33- CD14- gdTCR+
Natural Killer T cells (NKT)	CD3+ CD19- CD33- CD14- gdTCR- CD56+
CD4/CD8 Double-Negative T cells (DNT)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4- CD8-
CD4/CD8 Double-Positive T cells (DPT)	CD3+ CD19- CD33- CD14- gdTCR- CD56- CD4+ CD8+

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B cells	CD19+ CD3- CD11b- CD33- CD14-
Naive	CD19+ CD3- CD11b- CD33- CD14- CD38+ CD27-
Memory	CD19+ CD3- CD11b- CD33- CD14- CD38- CD27+
Plasmablasts (PB)	CD19+ CD3- CD11b- CD33- CD14- CD38hi CD27+
Dendritic Cells	CD3- CD19- CD56- CD14- CD16- HLA-DR+
Classical (cDC)	CD3- CD19- CD56- CD14- CD16- HLA-DR+ CD11c+ CD123-
Plasmacytoid (pDC)	CD3- CD19- CD56- CD14- CD16- HLA-DR+ CD11c- CD123+
Transitional (tDC)	CD3- CD19- CD56- CD14- CD16- HLA-DR+ CD11c+ CD123+
Monocytes	CD3- CD19- CD56-
Classical (cMono)	CD3- CD19- CD56- CD14+ CD16-
Intermediate (inMono)	CD3- CD19- CD56- CD14+ CD16+
Non-Classical (ncMono)	CD3- CD19- CD56- CD14- CD16+
Natural Killer (NK) cells	CD3- CD19- CD56+ CD14- CD33-
CD16+	CD3- CD19- CD56+ CD14- CD33- CD16+
CD16-	CD3- CD19- CD56+ CD14- CD33- CD16-
CD56+	CD3- CD19- CD56hi CD14- CD33- CD16-

Percentage of events in rare populations may not represent accurate values. If any population measured fewer than 100 cells in the population, they were excluded from validation analysis. Percentages of parent gates were evaluated for validation instead.

8.3 Materials and Methods

1. Instruments

- Helios mass cytometer (Serial numbers: 1070010393C and 1070011428C), by Standard BioTools.

2. Software

- CellEngine, version June 2023 (CellCarta, Montreal, Canada)
- CyTOF Software v7.1 (Standard BioTools, South San Francisco, California, USA)
- RStudio 4.2.3 (Posit, Boston, Massachusetts, USA)
- Premessa R script 0.3.4 on GitHub (San Francisco, California, USA)
- ggplot2 R package version: 3.4.2 on GitHub (San Francisco, California, USA)

3. Method

PBMC from healthy donors were fixed with PFA and stained with Teiko Bio's Pan-Immune Profiling Test consisting of metal-conjugated antibodies. Major immune cell populations such as T, B, NK and myeloid cells and their corresponding subpopulations

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were evaluated based on the combination of metal signals measured by mass cytometer.

4. Reagents

Table 2: List of critical reagents used

Material	Manufacturer	Catalog #
Ficoll-Paque PLUS	GE Healthcare	17-1440-03
Fetal Bovine Serum Heat Inactivated	ThermoFisher	16140071
DMSO	Sigma	D2650
Trypan Blue Dye 0.4% [2X stock]	Thermo Scientific	T10282
Countess™ Cell Counting Slide Chamber	Thermo Scientific	C10228
CryoClear 3001 Polypropylene Barcoded Cryogenic Vial	Globe Scientific (purchased from Amazon)	B008DI6IWK
1X PBS	Standard BioTools	201058
Hydrochloric acid (HCl), 37% (12N)	Fisher Scientific	A466
High purity palladium nitrate, equivalent of 10mg Pd-102, 103, 105, 106, 108, 110	Trace Sciences	Custom orders
Pierce Universal Nuclease for Cell Lysis 250 KU/mL [10,000X stock]	ThermoFisher	88700
10X Maxpar Barcode Perm (Saponin)	Standard BioTools	201057
10X eBioscience permeabilization buffer	Thermo Fisher	00-8333-56
Human TruStain FcX	BioLegend	422302
500 uM Iridium solution	Standard BioTools	201192B
Maxpar CAS Plus	Standard BioTools	201244
cis-Diammineplatinum(II) dichloride	Sigma Aldrich	P439425MG
RPMI 1640 Medium, with L-glutamine	Thermo Fisher	11875093
Penicillin-Streptomycin (5,000 U/mL) (100X)	Thermo Fisher	15070063

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16% Paraformaldehyde Aqueous Solution (diluted to 4%)	Electron Microscopy Sciences	15710
0.5M EDTA, pH 8.0 (100X)	Thermo Fisher	15575020
30% BSA	Sigma Aldrich	A728450ML
EQ Four Element Calibration Beads	Standard BioTools	201078

Note this list is not exhaustive; more details can be found in the referenced SOPs.

Table 3. List of antibodies used for Teiko Bio's Pan-Immune Profiling Test panel

Channel	Metal	Protein	Clone	Manufacturer	Catalog #	Lot #	Expiry
89	Y	CD45	HI30	BioLegend	304002	B283767	12/2028
112	Cd	CD8a	SK1	BioLegend	344702	B321682	01/2030
113	In	CD33	WM53	BioLegend	303438	B356506	06/2028
114	Cd	HLA-DR	L243	BioLegend	307602	B312523	09/2029
115	In	CD66b	G10F5	BioLegend	305102	B318093	11/2029
116	Cd	CD57	HNK-1	BioLegend	359602	B315946	12/2029
139	La	KLRG-1	SA231A2	BioLegend	367702	B322109	05/2029
141	Pr	CD3	UCHT1	BioLegend	300438	B335541	02/2030
142	Nd	CD19	HIB19	BioLegend	302202	B286428	01/2029
143	Nd	CD69	FN50	BioLegend	310902	B288994	06/2028
144	Nd	Granzyme B	GB11	BioRad	MCA2120	158956	10/2023
145	Nd	CD4	RPA-T4	BioLegend	300570	B324701	02/2028
146	Nd	CD11b	ICRF44	BioLegend	301302	B286270	10/2028
147	Sm	CD11c	Bu15	BioLegend	337202	B298273	03/2029
148	Nd	CD14	M5E2	BioLegend	301862	B339609	05/2028
149	Sm	TIGIT	MBSA43	ThermoFisher	16-9500-85	2059678	10/2023
150	Nd	CD86	IT2.2	BioLegend	305402	B330749	03/2029
151	Eu	CD123	6H6	BioLegend	306002	B254974	09/2027
152	Sm	gdTCR	B1	BioLegend	331236	B311994	01/2029
153	Eu	CD45RA	HI100	BioLegend	304102	B295482	12/2028
154	Sm	TIM3	F38-2E2	BioLegend	345010	B320064	05/2029
155	Gd	CD95	DX2	BioLegend	305656	B309317	03/2028

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156	Gd	PD-L1	29E.2A3	BioLegend	329702	B301673	11/2025
157	Gd	CCR7	G043H7	BioLegend	353202	B313274	02/2028
158	Gd	CD27	L128	BD Biosciences	624084	1315801	12/2023
159	Tb	CD39	A1	BioLegend	328202	B318457	12/2028
160	Gd	Tbet	4B10	BioLegend	644802	B335065	03/2029
161	Dy	CTLA-4	14D3	ThermoFisher	14-1529-82	2053204	10/2023
162	Dy	Foxp3	PCH101	ThermoFisher	14-4776-37	2440139	11/2023
163	Dy	CD28	L293	BD Biosciences	340975	41284673	11/2023
164	Dy	CD161	HP-3G10	BioLegend	339902	B321384	10/2029
165	Ho	CD127	R34-34	Standard BioTools	3165008B	2112313-09	2/2023
166	Er	CD74	LN2	BioLegend	326802	B317213	05/2028
167	Er	CD25	M-A251	BioLegend	356102	B332599	05/2028
168	Er	Ki67	Ki-67	BioLegend	350502	B247138	09/2027
169	Tm	ICOS	C398.4A	BioLegend	313502	B258672	11/2027
170	Er	LOX-1	15C4	BioLegend	358602	B264983	12/2024
171	Yb	CD15	W6D3	BioLegend	323002	B254011	11/2027
172	Yb	CD38	HIT2	BioLegend	303502	B285716	10/2027
173	Yb	IgG4	HP6025	Southern Biotech	9200-01	F0421-VC31	11/2022
174	Yb	PD-1	EH12.2H7	BioLegend	329902	B320065	07/2028
175	Lu	LAG-3	11C3C65	BioLegend	369302	B269396	06/2028
176	Yb	CD56	NCAM16.2	BD Biosciences	559043	9346558	10/2023
209	Bi	CD16	3G8	BioLegend	302002	B254382	10/2027

All experiments were completed before any antibodies expired.

5. Sample Processing

a. **Sample collection**

Blood product from 9 donors (subject IDs W070521202743, W070521202744, W070521411807, W070522016567, W070522116402, W070522116409, W070522002639, W070522002641 and W070522002642) were purchased from the Stanford Blood Bank (Palo Alto, CA, USA) as buffy coat or leukocyte

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reduction system (LRS) chambers. PBMCs were isolated using a Ficoll Density Gradient as described in DCS020 Isolation of PBMC by Ficoll and stored in liquid nitrogen until use.

b. Sample preparation

Sample preparation was performed as described in DCS007 CyTOF Staining with Barcode; buffers were prepared as described in DCS006 Preparation of CyTOF reagents. In short, cryopreserved PBMC samples were thawed into 10 mL of room temperature cell culture medium (RPMI-1640, 10% FBS, 1x L-glutamine, 1x penicillin/streptomycin) supplemented with 0.025 U/mL nuclease (Thermo Fisher). Cells were washed once with cell culture medium (centrifugation for 5 min, 350 g at RT) and then resuspended in PBS + 5mM EDTA at the density of 1 million cells per mL. Viability staining was performed by adding cisplatin to a final concentration of 25 μ M, followed by incubation for 1 min at RT, washed with 2X volume of PEB (PBS + 5mM EDTA + 0.5% BSA) and fixed with 1.6% PFA in PEB for 10 min at RT. Fixation was performed as described in DCS003 PBMC fixation with PFA. Fixed cells were pelleted by centrifugation for 5 min, 600 g at 4C, and washed twice with PEB before staining and frozen at -80C until staining.

c. Sample barcoding

For some experiments, samples were barcoded prior to antibody staining using a 20-Plex Pd Barcoding Kit (Standard BioTools) following manufacturer instructions and as described in DCS007 CyTOF Staining with Barcode. Briefly, after counting, 1 million cells were washed with Maxpar Barcode Perm Buffer (Standard BioTools) twice and barcoded with unique combinations of Pd isotopes for 30 min at room temperature on a shaker in Maxpar Barcode Perm Buffer. Cells were washed twice with eBioscience perm buffer and once with Maxpar Cell Staining Buffer (CSB, Standard BioTools) and pooled into a single tube.

d. Antibody staining

Antibody staining was performed as described in DCS007 CyTOF Staining with Barcode; buffers were prepared as described in DCS006 Preparation of CyTOF reagents. A staining antibody cocktail was prepared fresh in CSB according to previously determined titers at 25 μ L of staining volume per 1 million cells. The pooled barcoded sample was stained for 10 min at room temp with Fc receptor blocking solution (BioLegend) followed by 1h incubation with staining antibody cocktail at RT. PBMC were stained using a panel of 44 antibodies (Table 3). Cells were washed twice with CSB and resuspended in intercalation solution (4% PFA in PBS and 0.5 mM iridium intercalator (Standard BioTools) for 20 min at RT. Samples were pelleted, resuspended in a small volume of CSB and frozen at -80C until the day of acquisition.

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e. Data acquisition

As described in DCS007 CyTOF Staining with Barcode, before acquisition, samples were washed once in CSB and twice in CAS Plus (Standard BioTools) and filtered through a cell strainer (Falcon). Buffers were prepared as described in DCS006 Preparation of CyTOF reagents. Cells were then resuspended at 0.7 million cells/mL in CAS-Plus supplemented with EQ4 element calibration beads (Standard BioTools) and acquired on a Helios mass cytometer (Standard BioTools).

f. Data analysis

Manual gating of FCS files was performed using CellEngine (CellCarta, Montreal, Canada). Cell populations were gated based on the parameters described in Table 1 and as shown in Figure 1. Frequencies were determined as % of total live non-granulocytes. Populations with under 100 cells were excluded from the analysis to avoid inaccurate measurements caused by low-frequency populations; only populations where a defined number of samples met the 100 cell minimum were retained for further analysis. The cut-off is specified in the section for each experiment.

For precision analyses, each population as a percentage of non-granulocytes (i.e. their frequency) was compared to its corresponding populations across samples, and a coefficient of variation (CV) was calculated as a percentage: $\%CV = 100 * \text{standard deviation}(\text{frequency}) / \text{mean}(\text{frequency})$. The precision assessment data were visualized with R using the ggplot2 package to display the %CV vs. the mean cell count of the population.

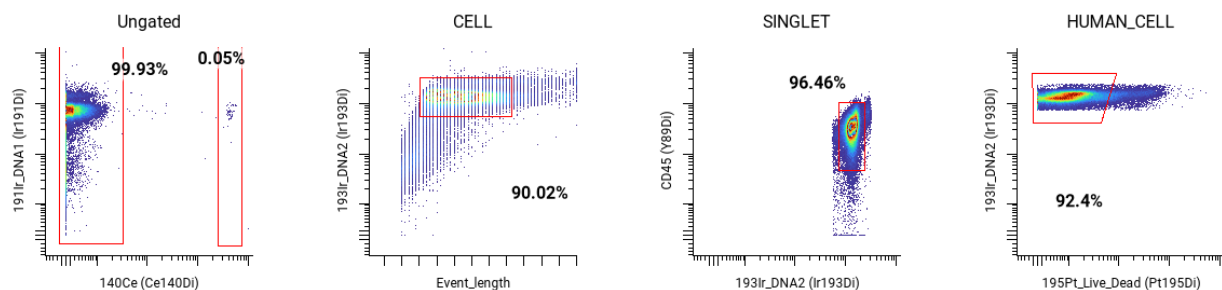
For stability assessments, the percent change between D22 and D1 was calculated as follows: $\% \text{ change} = 100 * (\text{frequency at D22} - \text{frequency at D1}) / \text{frequency at D1}$. The data were visualized with R using the ggplot2 package to display the median percent change between D1 and D22, along with an error bar extending above and below the median between the following values: (median percent change - standard error of the mean [SEM]) to (median percent change + SEM). The SEM was calculated as follows: $SEM = SD(\text{percent change between D1 and D22}) / \sqrt{\text{number of samples}}$.

For the reference range experiment, we calculated the median, standard deviation, minimum, and maximum percent of frequency for each population.

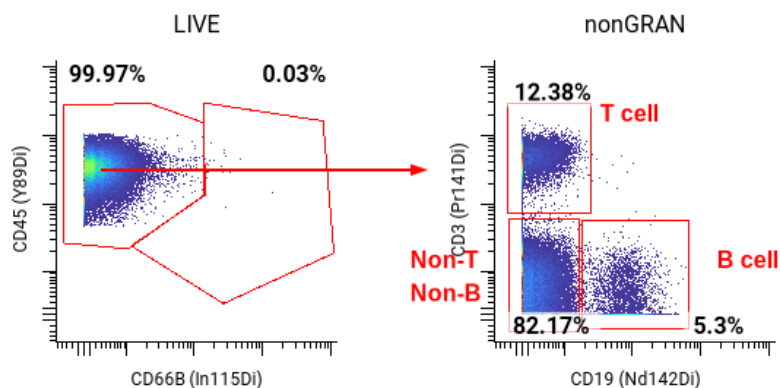
Figure 1: Gating strategy used for Teiko Bio's Pan-Immune Profiling Test

A. Gating strategy for live cells (from total events)

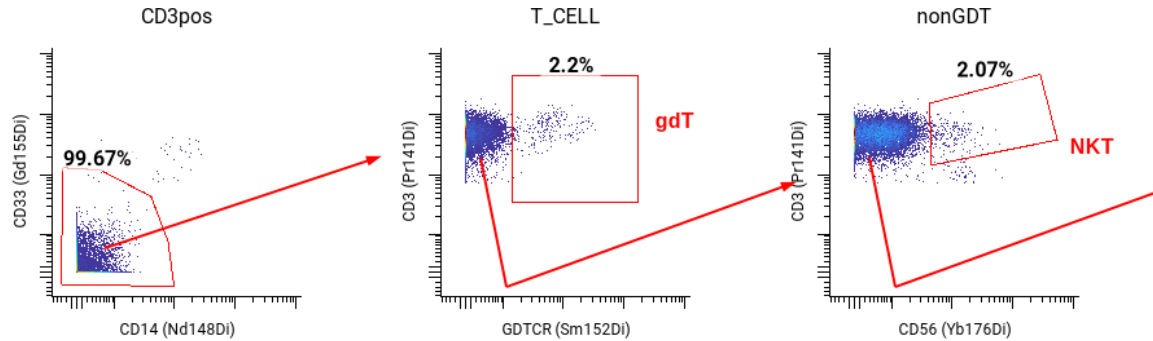
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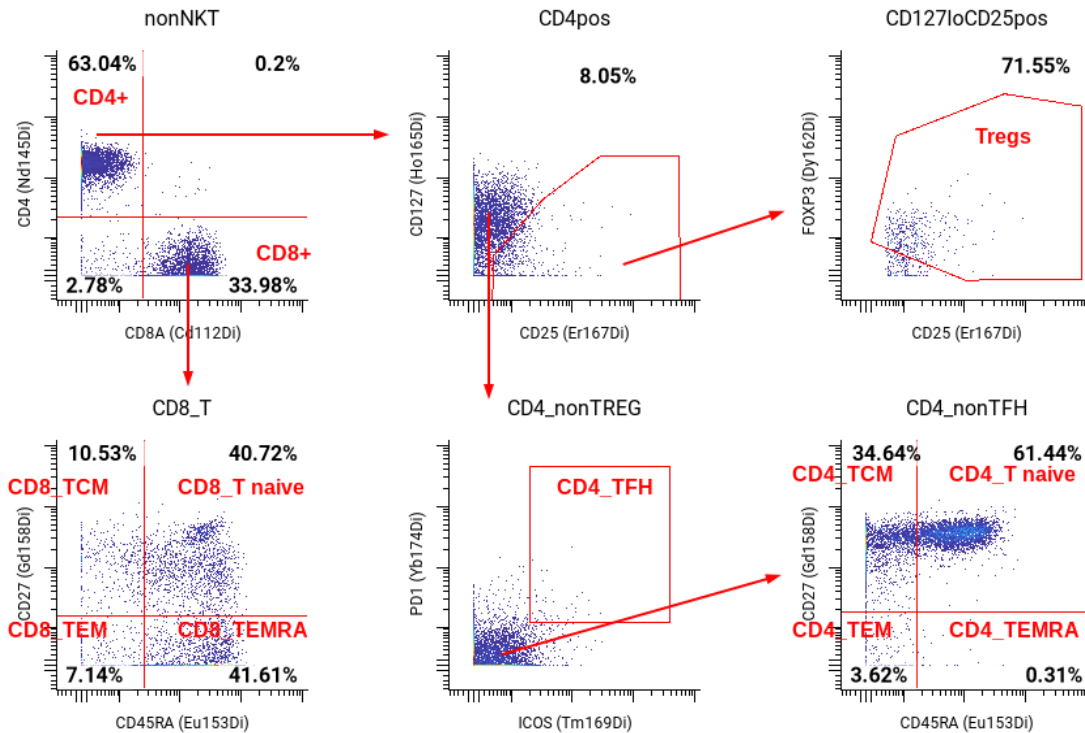
B. Gating strategy for non-granulocytes (from live cells)



C. Gating strategy for T cells (from non-granulocytes)

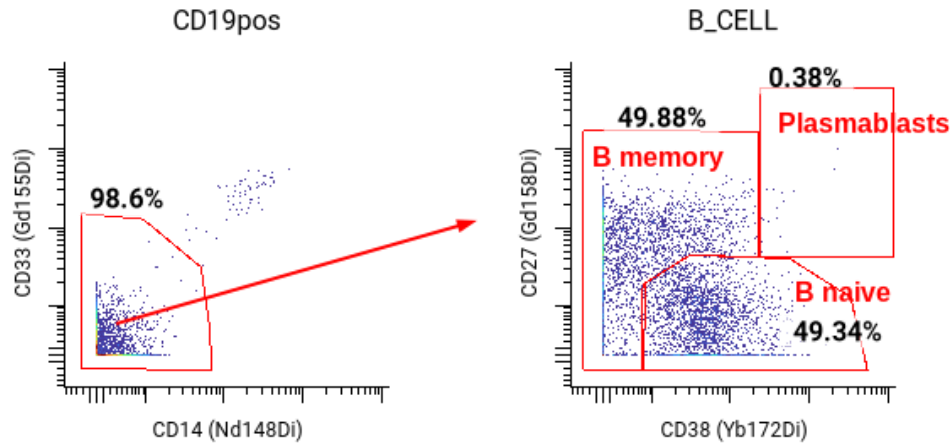


Continuation from non-NKT gate

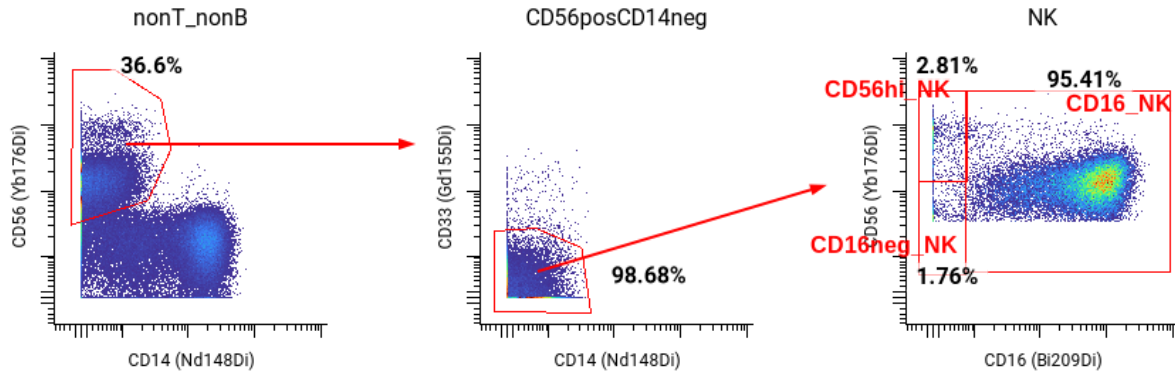


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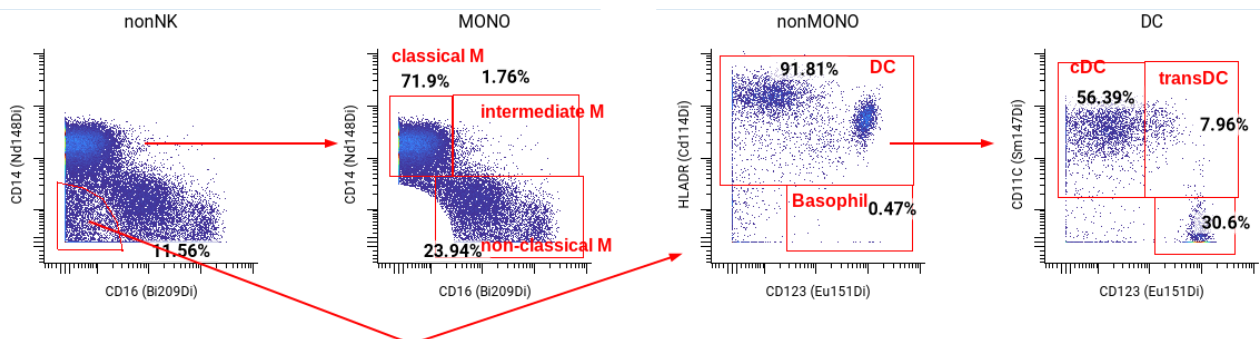
D. Gating strategy for B cells (from non-T cells)



E. Gating strategy for NK cells (from non-T and non-B cells)



F. Gating strategy for monocytes and dendritic cells (from non-T, non-B and non-NK cells)



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8.4 Validation Parameters

The purpose of this validation is to describe the process and requirements for Teiko Bio's Pan-Immune Profiling test.

1. Assay Optimization and Accuracy

Assay optimization was performed to ensure optimal channel selection, optimal antibody concentration and minimal signal spillover into other CyTOF channels for all markers on the test. We first determined optimal channel selection based on antigen abundance and instrument detection efficiency for the metal isotope tag. Next, we determined antibody concentration by testing five different antibody concentrations in a serial dilution: 6 µg/mL, 3 µg/mL, 1.5 µg/mL, 0.75 µg/mL, 0.375 µg/mL, and 0.1875 µg/mL. Depending on the nature of the marker, healthy cells were either unstimulated or stimulated with an appropriate biological stimulus, such as PHA or PMA. Depending on the obtained expression signal, ideal antibody concentration was determined by either assessing the stain index or the arcsinh ratio. Finally, spillover was assessed by looking at signals in other channels. To confirm the panel is able to detect all major immune cell populations, accuracy was assessed by determining frequencies of all major immune cell lineages and subsets in total PBMCs from 8 healthy subjects (W070521202743, W070521202744, W070522016567, W070522116402, W070522116409, W070522002639, W070522002641 and W070522002642).

2. Precision

Intra-run, Inter-run, and Inter-operator precision was evaluated to ensure test results are not affected by technical intra-run or inter-run variables, or by operator variables during the fixation step.

- a. Intra-run precision was assessed by taking cryopreserved PBMCs from one (1) healthy donor (W070521411807), splitting the sample into three technical replicates, and processing them all together in the same manner. The process was performed by the same operator, who ran all samples together on the same CyTOF machine during one run. Variance between replicates was calculated for precision analysis. Acceptance criteria were $\leq 25\%$ CV between replicates.
- b. Inter-run precision was assessed by taking cryopreserved PBMCs from one (1) healthy donor (W070521202744) and splitting the sample into three technical replicates. Each replicate was fixed on the same day, but stained and run on separate days. One operator performed all steps. Samples were stained and run on 2022-10-18, 2022-11-02 and 2022-11-03, respectively. Variance between days was calculated for precision analysis. Acceptance criteria were $\leq 30\%$ CV between individual runs.

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- c. Inter-operator precision for fixation was assessed by taking cryopreserved PBMCs from two (2) healthy donors (W070521411807 and W070521202743) and splitting the samples into two replicates. Two different operators (M.M. and L.E.) fixed one replicate from each donor. Note that one (1) operator subsequently stained and ran all samples. Variance between operator-processed replicates was calculated for precision analysis. Acceptance criteria were $\leq 25\%$ CV between operators.

3. Stability

Stability was evaluated to ensure test results are not affected by storage-related variables. Stability was assessed by taking cryopreserved PBMCs from 4 (four) healthy donors (W070522002642, W070522002641, W070522002639 and W070522116409) and fixing and staining them by the same operator on the same day, 2022-12-21. Then, samples were split into two replicates, and all were stored at -80C. One set of replicates was thawed and run on CyTOF on 2022-12-22, one day after staining (D1). The other set of replicates was thawed and run on CyTOF on 2023-01-12 (D22). All steps were performed by the same operator. Percentage of change in immune population frequencies between days was calculated for stability analysis. Acceptance criteria were $\leq 25\%$ change in immune population frequencies between D1 and D22.

4. Reference Range

Reference intervals were established using a subset of healthy donors to determine baseline frequencies of all major immune cell lineages and subsets . Reference ranges were established by taking cryopreserved PBMCs from 8 (eight) healthy donors (W070521202743, W070521202744, W070522016567, W070522116402, W070522116409, W070522002639, W070522002641 and W070522002642) and fixing and staining them by the same operator on the same day, and running on CyTOF on the next day. All steps were performed by the same operator. Donors were of unknown sex and age. Median, standard deviation and range was calculated for each major immune population.

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8.5 Results

1. Method Optimization and Accuracy

Method optimization was performed to select the optimal channels, metals, and concentrations for each marker. Optimized settings for each marker can be seen in Table 4.

Table 4: Teiko Bio's Pan-Immune Profiling panel with channels, metals and antibody concentrations.

Channel	Metal	Protein	Clone	Optimal Concentration (µg/mL)	Stock Concentration (mg/mL)	Volume per 100 µL test
89	Y	CD45	HI30	1.5	0.3	0.5
112	Cd	CD8a	SK1	1.5	0.5	0.3
113	In	CD33	WM53	0.1875	0.3	0.0625
114	Cd	HLA-DR	L243	0.375	0.3	0.125
115	In	CD66b	G10F5	0.75	0.3	0.25
116	Cd	CD57	HNK-1	0.1875	0.3	0.0625
139	La	KLRG-1	SA231A2	0.1875	0.3	0.0625
141	Pr	CD3	UCHT1	0.75	0.3	0.25
142	Nd	CD19	H1B19	0.75	0.3	0.25
143	Nd	CD69	FN50	0.375	0.3	0.125
144	Nd	Granzyme B	GB11	0.375	0.3	0.125
145	Nd	CD4	RPA-T4	0.375	0.3	0.125
146	Nd	CD11b	ICRF44	0.75	0.3	0.25
147	Sm	CD11c	Bu15	0.1875	0.3	0.0625
148	Nd	CD14	M5E2	0.75	0.3	0.25
149	Sm	TIGIT	MBSA43	0.375	0.3	0.125
150	Nd	CD86	IT2.2	0.75	0.3	0.25
151	Eu	CD123	6H6	0.375	0.3	0.125
152	Sm	gdTCR	B1	1.5	0.3	0.5
153	Eu	CD45RA	HI100	0.75	0.3	0.25
154	Sm	TIM3	F38-2E2	1.5	0.3	0.5
155	Gd	CD95	DX2	0.375	0.3	0.125

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156	Gd	PD-L1	29E.2A3	0.375	0.3	0.125
157	Gd	CCR7	G043H7	3	0.3	1
158	Gd	CD27	L128	0.1875	0.3	0.0625
159	Tb	CD39	A1	0.75	0.3	0.25
160	Gd	Tbet	4B10	1.5	0.3	0.5
161	Dy	CTLA-4	14D3	0.375	0.3	0.125
162	Dy	Foxp3	PCH101	0.75	0.3	0.25
163	Dy	CD28	L293	0.375	0.3	0.125
164	Dy	CD161	HP-3G10	0.75	0.3	0.25
165	Ho	CD127	R34-34	0.375	0.3	0.125
166	Er	CD74	LN2	0.375	0.3	0.125
167	Er	CD25	M-A251	0.1875	0.3	0.0625
168	Er	Ki67	Ki-67	0.75	0.3	0.25
169	Tm	ICOS	C398.4A	0.375	0.3	0.125
170	Er	LOX-1	15C4	0.1875	0.3	0.0625
171	Yb	CD15	W6D3	0.75	0.3	0.25
172	Yb	CD38	HIT2	0.1875	0.15	0.125
173	Yb	IgG4	HP6025	0.75	0.3	0.25
174	Yb	PD-1	EH12.2H7	0.1875	0.3	0.0625
175	Lu	LAG-3	11C3C65	0.375	0.3	0.125
176	Yb	CD56	NCAM16.2	0.1875	0.15	0.125
209	Bi	CD16	3G8	1.5	0.3	0.5

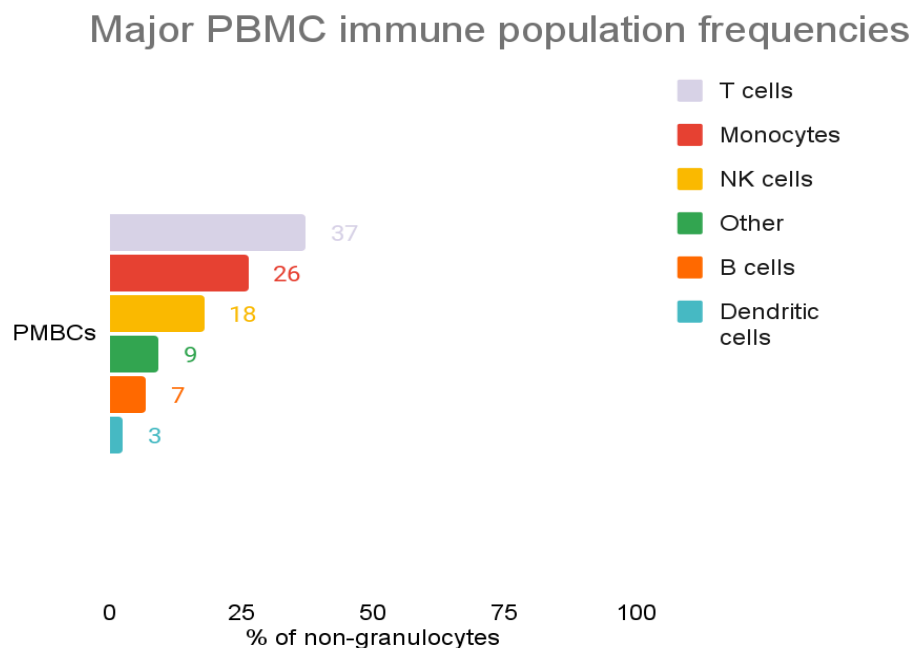
All individual data files showing the stain index or arcsinh ratio, and spillover assessments, can be found in DCS122 Appendix A Teiko Pan-Immune Profiling Panel Antibody Titration Data. We were able to find an optimal concentration for all antibodies in the panel, and we observed minimal to no spillover for all markers in other channels.

To determine accuracy, we applied this panel to PBMCs from 8 healthy subjects to establish the test's ability to detect all individual major immune cell populations from these mixed samples. We were able to detect all major immune cell populations and subsets within T cells, B cells, NK cells, monocytes and dendritic cells in PBMCs from 8 healthy subjects. Figures 2-7 show data averaged across the 8 donors. Cell populations for which individual subjects had fewer than 100 detectable events were excluded from the average. Data for individual donors can be found in

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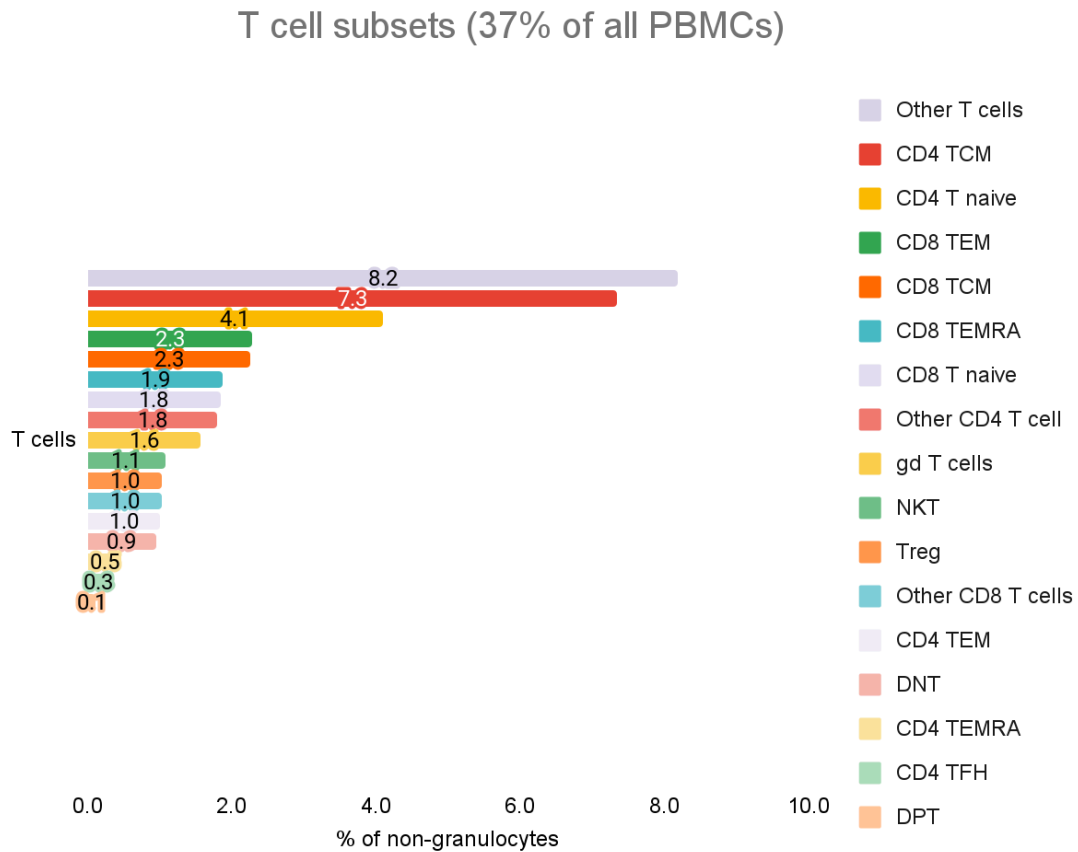
DCS122 Appendix B Teiko Bio Pan-Immune Profiling Test Validation Report Data. The only population for which none of the subjects had sufficient cells (sufficient events to pass limit of detection) were plasmablasts.

Figure 2: Median frequency of major immune lineages from PBMCs of healthy subjects across all 8 donors.



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Figure 3: Median of individual T cell subset frequencies in PBMCs across all 8 donors.

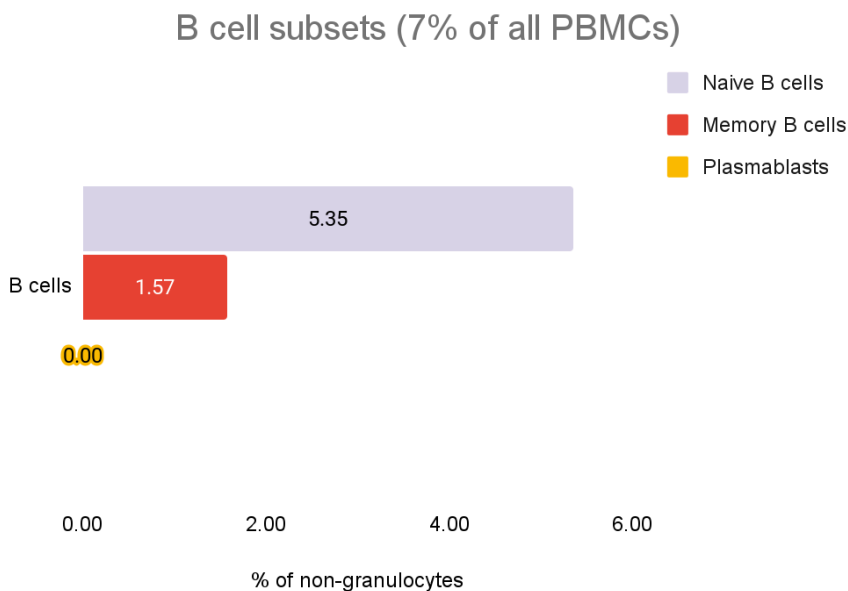


Note that the following populations have fewer donor samples due to populations < 100 events:

- CD4+ TFH (n=7)
- CD4+ TEMRA cells (n=2)
- CD8+ TEM (n=6)
- CD8+ TEMRA (n=6)
- NKT cells (n=7)
- DPT (n=5)

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Figure 4: Median of individual B cell subset frequencies in PBMCs across all 8 donors.



Note that in this experiment, none of the donors had plasmablasts >100 events.

Figure 5: Median of individual NK cell subset frequencies in PBMCs across all 8 donors.

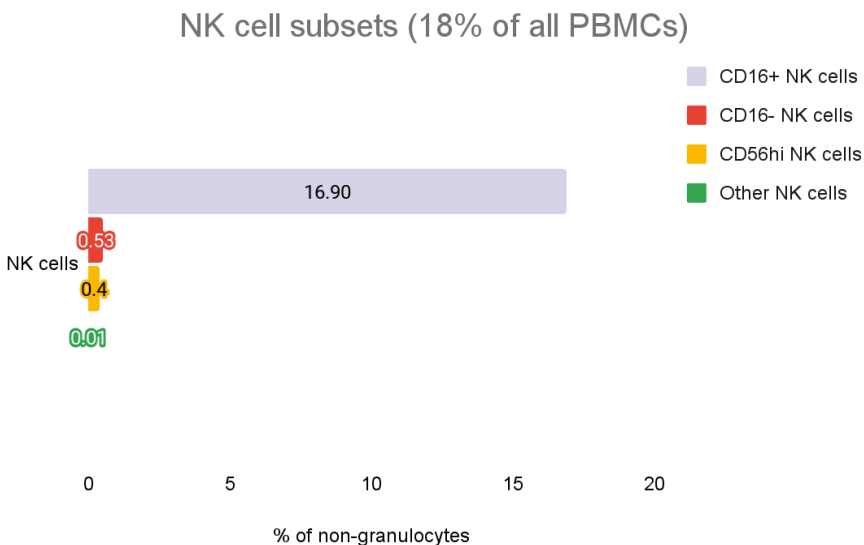


Figure 6: Median of individual monocyte subset frequencies in PBMCs across all 8 donors.

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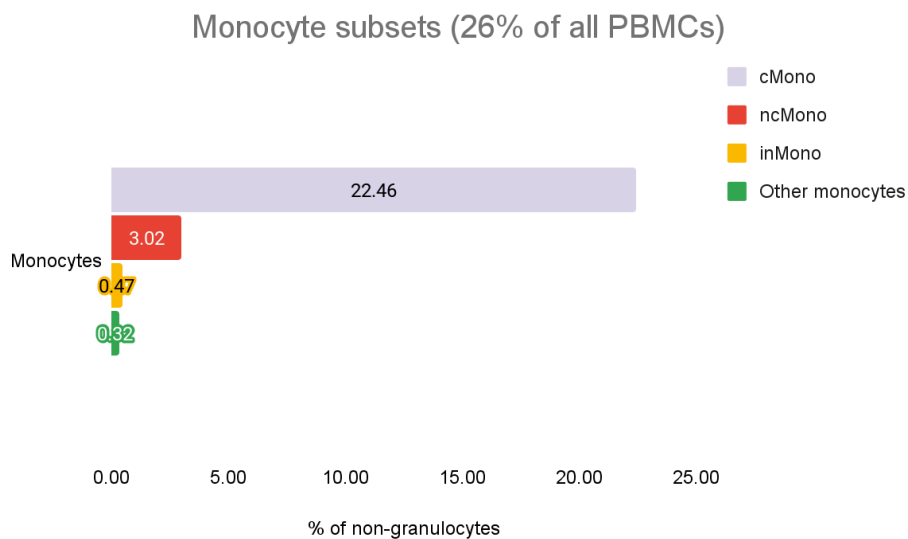
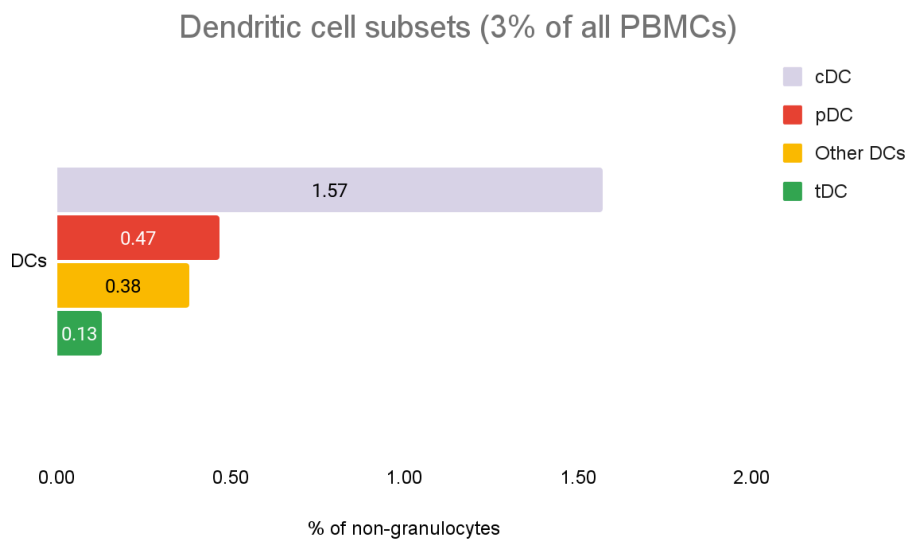


Figure 7: Median of individual dendritic cell subset frequencies in PBMCs across all 8 donors.



Note that tDCs only represent n=3 donors due to low event count in the other donors.

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2. Precision

a. Intra-Run

Intra-run precision was assessed by taking cryopreserved PBMCs from one (1) healthy donor (1807), splitting the sample into three technical replicates, and processing them all together in the same manner. The process was performed by the same operator, who ran all samples together on the same CyTOF machine during one run. Variance between replicates was calculated for precision analysis and can be seen in Table 5 and Figure 8. All measurable (>100 cells median) cell populations passed the acceptable threshold of $\leq 25\%$ variation between replicates.

Table 5: Summary of Intra-Run Precision Assessment

Cell Subset	# Subjects	Median # of cells measured	Average % of non-granulocytes	Coefficient of Variation between replicates (%)
T cells	3	82182	53.79	1.27
CD4+ T cells	3	37869	24.92	0.95
Naive	3	14054	9.15	1.33
TFH	3	281	0.18	2.69
Treg	3	2065	1.38	3.04
TCM	3	18566	12.17	1.73
TEM	3	2136	1.39	0.87
TEMRA	0*	*	*	*
CD8+ T cells	3	32855	21.42	1.62
Naive	3	18493	12.12	0.63
TCM	3	9344	5.98	3.35
TEM	3	2702	1.73	2.64
TEMRA	3	2493	1.60	4.97
$\gamma\delta$ T cells	3	4994	3.23	2.38
NKT cells	3	4418	2.83	4.52
DNT	3	1782	1.14	3.05
DPT	3	361	0.24	3.67
B cells	3	17785	11.30	2.37
Naive	3	11880	7.51	2.85

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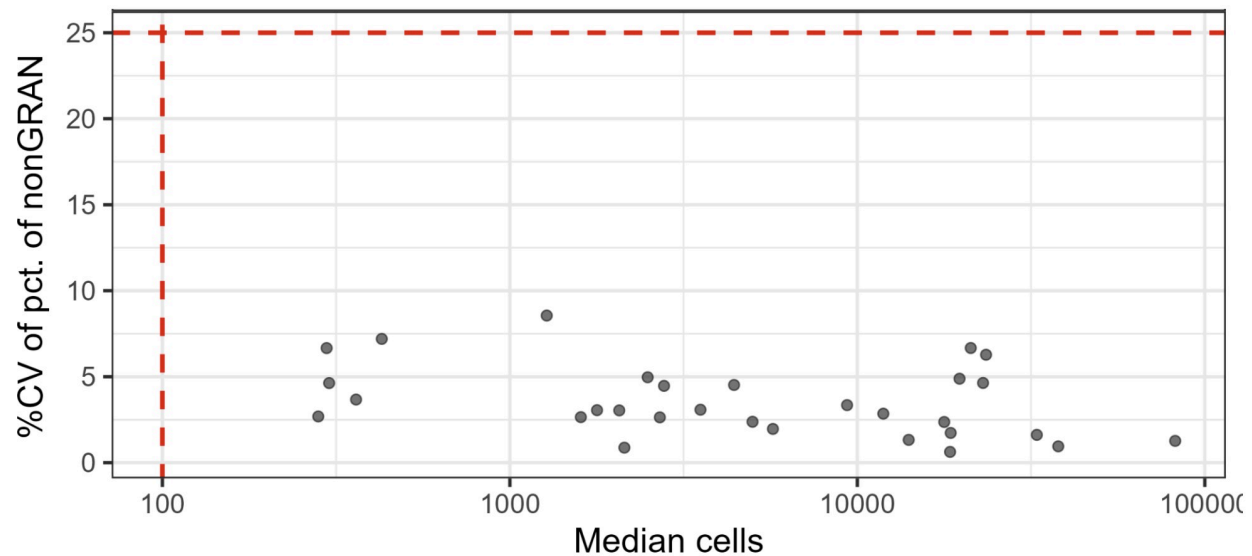
Memory	3	5716	3.68	1.96
PB	0*	*	*	*
Dendritic Cells	3	3537	2.35	3.08
cDC	3	1277	0.87	8.55
pDC	3	297	0.18	6.66
tDC	0*	*	*	*
Monocytes	3	23466	15.82	6.28
cMono	3	21207	14.33	6.67
inMono	3	302	0.20	4.63
ncMono	3	1601	1.06	2.65
NK cells	3	23004	14.74	4.64
CD16+	3	19688	12.64	4.88
CD16-	3	2778	1.78	4.47
CD56+	3	428	0.27	7.20
Total average				3.52

**Populations with fewer than 100 cell events were excluded from analysis. Populations in which fewer than 3 replicates had sufficient cell count for analysis were excluded from precision analysis and CV calculations. As a result, we excluded CD4+ TEMRA cells, Plasmablasts and Transitional Dendritic Cells from analysis.*

Data for individual replicates can be found in DCS122 Appendix B Teiko Bio Pan-Immune Profiling Test Validation Report Data.

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Figure 8: Coefficient of Variation in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Intra-Run Precision Assessment



b. Inter-Run

Inter-run precision was assessed by taking cryopreserved PBMCs from one (1) healthy donor (2744) and splitting the sample into three technical replicates. Each replicate was fixed on the same day, but stained and run on separate days. One operator performed all steps. Samples were stained and run on 2022-10-18, 2022-11-02 and 2022-11-03, respectively. Variance between days was calculated for precision analysis and can be seen in Table 6 and Figure 9. All measurable (>100 cells median) cell populations passed the acceptable threshold of $\leq 30\%$ variation between replicates, which were measured on different days (and thus different runs).

Table 6: Summary of Inter-Run Precision Assessment

Cell Subset	# Subjects	Median # of cells measured	Average % of non-granulocytes	Coefficient of Variation between replicates (%)
T cells	3	161678	39.86	0.39
CD4+ T cells	3	95849	23.50	0.86
Naive	3	31813	7.78	3.45
TFH	3	2806	0.74	11.40

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Treg	3	5825	1.39	3.24
TCM	3	44597	10.73	4.46
TEM	3	9978	2.39	5.25
TEMRA	3	247	0.06	29.73
CD8+ T cells	3	45445	11.31	1.15
Naive	3	5390	1.33	5.36
TCM	3	11714	2.91	1.09
TEM	3	19020	4.41	7.84
TEMRA	3	10746	2.67	9.97
γδ T cells	3	12754	3.20	2.21
NKT cells	3	2804	0.65	7.99
DNT	3	4563	1.09	2.88
DPT	3	519	0.12	9.39
B cells	3	69982	17.28	0.25
Naive	3	53539	13.23	0.87
Memory	3	16122	3.96	2.00
PB	3	251	0.05	16.15
Dendritic Cells	3	7648	1.82	10.80
cDC	3	3505	0.84	14.93
pDC	3	591	0.15	14.77
tDC	3	118	0.03	5.84
Monocytes	3	84660	20.65	1.64
cMono	3	69074	17.01	0.88
inMono	3	486	0.13	16.09
ncMono	3	9974	2.41	10.47
NK cells	3	75699	18.87	1.25
CD16+	3	71193	17.71	1.95
CD16-	3	3589	0.87	11.75

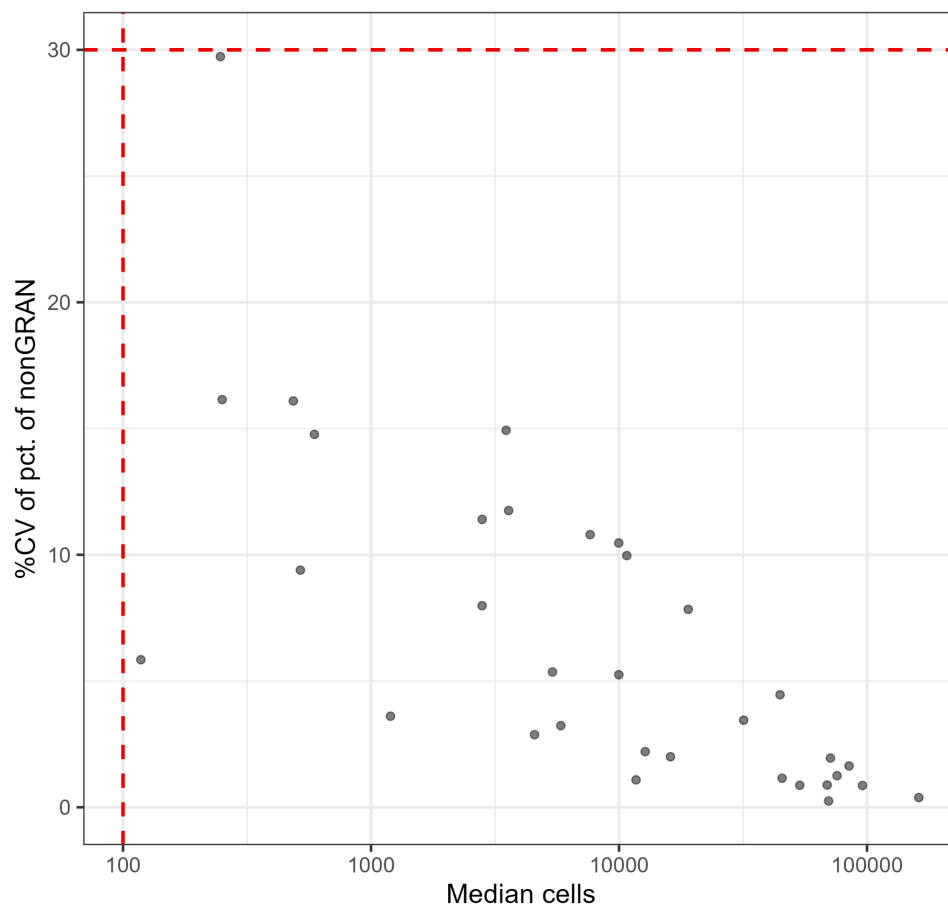
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CD56+	3	1198	0.28	3.61
Total average				6.66

**Populations with fewer than 100 cell events were excluded from analysis. Populations in which fewer than 3 replicates had sufficient cell count for analysis were excluded from precision analysis and CV calculations. No populations were excluded from analysis in this assessment.*

Data for individual replicates can be found in DCS122 Appendix B Teiko Bio Pan-Immune Profiling Test Validation Report Data.

Figure 9: Coefficient of Variation in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Inter-Run Precision Assessment



c. Inter-Operator Fixation

Inter-operator fixation precision for fixation was assessed by taking cryopreserved PBMCs from two (2) healthy donors (1807 and 2743) and splitting

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the samples into two replicates. Two different operators (M.M. and L.E.) fixed one replicate from each donor. Note that one (1) operator subsequently stained and ran all samples. Variance between operator-processed replicates was calculated for precision analysis and can be seen in Tables 7 , 8 and Figure 10. All measurable (>100 cells median) cell populations passed the acceptable threshold of $\leq 25\%$ variation between operators.

Table 7: Summary of Inter-Operator Fixation Precision Assessment - Subject 1

Cell Subset - Healthy subject 1	# Subjects	Median # of cells measured	% of non-granulocytes operator 1	% of non-granulocytes operator 2	Coefficient of Variation between operators (%)
T cells	1	39566	48.20	48.96	1.11
CD4+ T cells	1	18349	22.33	22.72	1.23
Naive	1	6548	7.73	8.26	4.71
TFH	1	257	0.31	0.32	2.81
Treg	1	1357	1.69	1.65	1.63
TCM	1	8649	10.66	10.63	0.21
TEM	1	1094	1.38	1.32	2.90
TEMRA	0*	*	*	*	*
CD8+ T cells	1	15695	19.21	19.36	0.57
Naive	1	8969	10.66	11.27	3.90
TCM	1	3955	5.14	4.69	6.50
TEM	1	1204	1.50	1.47	1.62
TEMRA	1	1568	1.91	1.94	1.32
$\gamma\delta$ T cells	1	2189	2.65	2.72	1.78
NKT cells	1	2039	2.32	2.63	8.81
DNT	1	1069	1.45	1.23	12.08
DPT	1	226	0.24	0.30	17.74
B cells	1	7759	10.05	9.22	6.06
Naive	1	4952	6.44	5.87	6.60
Memory	1	2731	3.51	3.26	5.19

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PB	0*	*	*	*	*
Dendritic Cells	1	1267	1.58	1.54	1.55
cDC	1	844	1.00	1.06	4.64
pDC	0*	*	*	*	*
tDC	*	*	*	*	*
Monocytes	1	18242	23.23	21.93	4.09
cMono	1	16934	21.61	20.32	4.35
inMono	1	488	0.58	0.61	4.59
ncMono	1	762	0.98	0.91	5.59
NK cells	1	12490	14.82	15.71	4.12
CD16+	1	12028	14.27	15.13	4.09
CD16-	1	190	0.20	0.26	18.89
CD56+	1	268	0.33	0.33	1.42
Total average					4.83

**Populations with fewer than 100 cell events were excluded from analysis. As a result, we excluded CD4+ TEMRA cells, Plasmablasts, Plasmacytoid Dendritic Cells and Transitional Dendritic Cells from analysis.*

Table 8: Summary of Inter-Operator Fixation Precision Assessment - Subject 2

Cell Subset - Healthy subject 2	# Subjects	Median # of cells measured	% of non-granulocytes operator 1	% of non-granulocytes operator 2	Coefficient of Variation between operators (%)
T cells	1	21161	19.21	21.04	6.40
CD4+ T cells	1	13542	11.91	13.85	10.61
Naive	1	3428	2.74	3.79	22.72
TFH	1	401	0.35	0.41	12.03
Treg	1	932	0.78	0.99	16.26
TCM	1	7990	7.30	7.90	5.54
TEM	1	536	0.54	0.48	7.10
TEMRA	0*	*	*	*	*
CD8+ T cells	1	5065	4.75	4.88	1.96

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Naive	1	2119	1.88	2.15	9.49
TCM	1	2731	2.65	2.54	2.92
TEM	0*	*	*	*	*
TEMRA	1	164	0.17	0.14	15.46
γδ T cells	1	1326	1.34	1.18	9.05
NKT cells	1	263	0.25	0.25	0.82
DNT	1	724	0.72	0.66	5.70
DPT	1	243	0.25	0.21	9.66
B cells	1	4619	4.74	4.04	11.25
Naive	1	3601	3.70	3.15	11.27
Memory	1	985	1.01	0.86	10.79
PB	0*	*	*	*	*
Dendritic Cells	1	2432	2.30	2.32	0.67
cDC	1	1433	1.35	1.37	1.24
pDC	1	730	0.68	0.71	3.75
tDC	0*	*	*	*	*
Monocytes	1	67071	64.62	62.93	1.87
cMono	1	64741	62.34	60.78	1.79
inMono	1	535	0.50	0.52	2.67
ncMono	1	1371	1.38	1.23	8.37
NK cells	1	7926	7.41	7.66	2.36
CD16+	1	6713	6.34	6.43	0.97
CD16-	1	416	0.36	0.43	13.47
CD56+	1	770	0.70	0.77	7.16
Total average					7.36

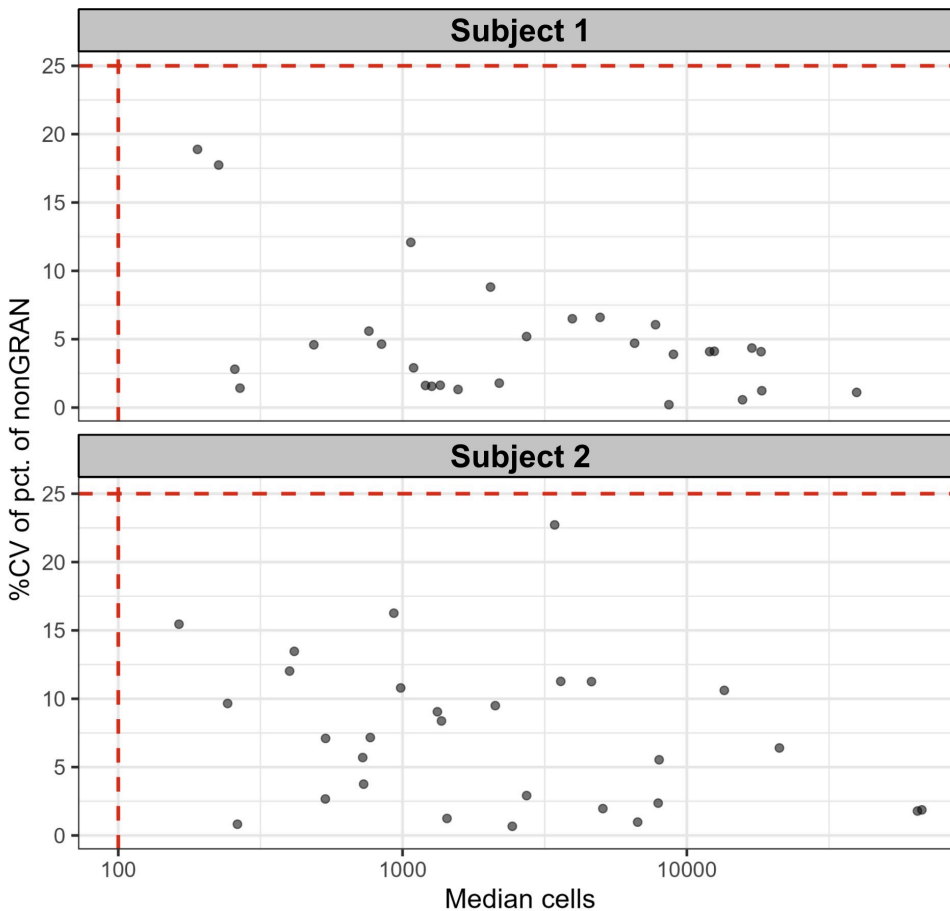
**Populations with fewer than 100 cell events were excluded from analysis. As a result, we excluded CD4+ TEMRA cells, CD8+ TEM Cells, Plasmablasts and Transitional Dendritic Cells from analysis.*

Data for individual replicates can be found in DCS122 Appendix B Teiko Bio

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Figure 10: Coefficient of Variation in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Inter-Operator Fixation Precision Assessment



3. Stability

Stability was evaluated to ensure test results are not affected by storage-related variables. Stability was assessed by taking cryopreserved PBMCs from 4 (four) healthy donors (W070522002642, W070522002641, W070522002639 and W070522116409) and fixing and staining them by the same operator on the same day, 2022-12-21. Then, samples were split into two replicates, and all were stored at -80C. One set of replicates was thawed and run on CyTOF on 2022-12-22, one day after staining (D1). The other

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set of replicates was thawed and run on CyTOF on 2023-01-12 (D22). All steps were performed by the same operator. Percentage of change in immune population frequencies between days was calculated for stability analysis and can be seen in Table 9 and Figure 11. All measurable (>100 cells median) cell populations passed the acceptable threshold of $\leq 25\%$ change between days.

Table 9: Summary of Stability Assessment

Cell Subset	# Subjects	Median # of cells D1	Median # of cells D22	Average % of non-granulocytes D1	Average % of non-granulocytes D22	Change between days (%)
T cells	4	56709	67349	32.98	33.51	1.46
CD4+ T cells	4	27227	33077	17.17	17.45	1.43
Naive	4	5038	6371	5.80	5.94	5.12
TFH	4	600	678	0.34	0.33	-4.50
Treg	4	1403	1764	1.16	1.21	4.74
TCM	4	8347	9873	6.21	6.27	0.36
TEM	4	2600	3081	2.54	2.56	-0.20
TEMRA	2*	*	*	*	*	*
CD8+ T cells	4	13420	16031	9.31	9.51	2.10
Naive	4	3191	3986	2.05	2.10	2.31
TCM	4	3197	3858	1.81	1.86	2.40
TEM	3	6002	7234	3.35	3.43	1.58
TEMRA	4	3221	3817	2.93	2.96	2.30
$\gamma\delta$ T cells	4	1500	1708	1.69	1.69	-0.31
NKT cells	4	4922	6039	3.50	3.57	2.41
DNT	4	2107	2337	1.23	1.19	-2.08
DPT	2*	*	*	*	*	*
B cells	4	8731	10248	6.27	6.36	1.22
Naive	4	6740	7992	4.83	4.93	2.05
Memory	4	1932	2190	1.39	1.38	-0.84
PB	0*	*	*	*	*	*

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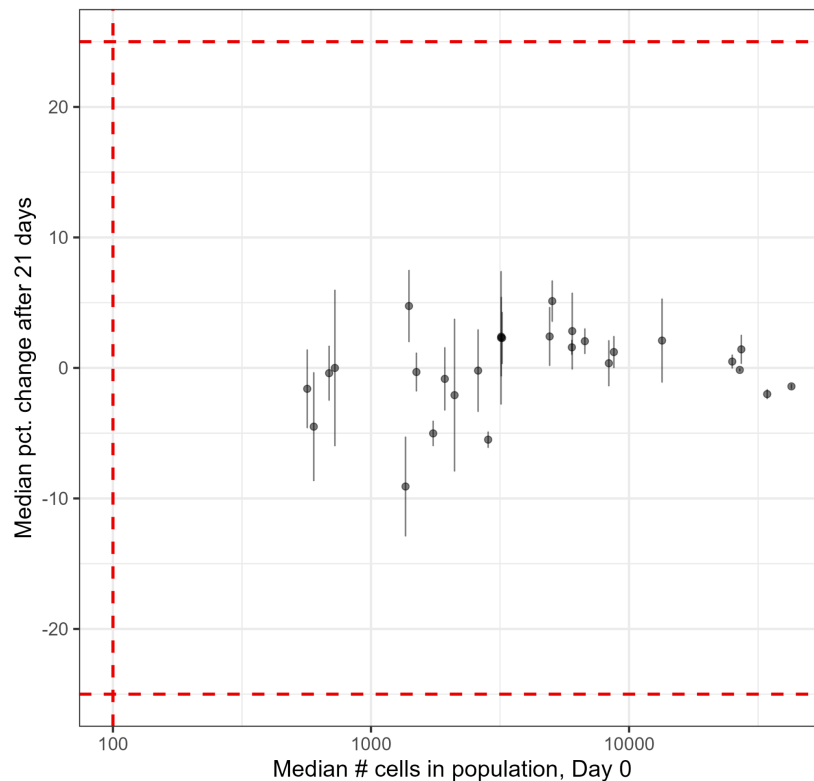
Dendritic Cells	4	2844	3148	2.22	2.11	-5.50
cDC	4	1741	1904	1.32	1.25	-5.02
pDC	4	688	789	0.44	0.44	-0.40
tDC	2*	*	*	*	*	*
Monocytes	4	42570	50453	32.31	31.88	-1.41
cMono	4	34292	40379	27.02	26.62	-2.00
inMono	4	725	885	0.53	0.57	0.00
ncMono	4	6022	7009	4.19	4.24	2.82
NK cells	4	26852	31007	24.47	24.45	-0.15
CD16+	4	25102	29103	23.10	23.22	0.49
CD16-	4	1361	1444	0.93	0.81	-9.09
CD56+	4	566	637	0.40	0.38	-1.60
Total average						-0.01

**Populations with fewer than 100 cell events were excluded from analysis. Populations in which fewer than 3 samples had sufficient cell count for analysis were excluded from stability analysis and change calculations. As a result, we excluded CD4+ TEMRA cells, CD4/CD8 Double-Positive T Cells, Plasmablasts and Transitional Dendritic Cells from analysis.*

Data for individual donors can be found in DCS122 Appendix B Teiko Bio Pan-Immune Profiling Test Validation Report Data.

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Figure 11: Median Percentage of Change in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Stability Assessment



4. Reference Range

Reference intervals were established by taking cryopreserved PBMCs from 8 (eight) healthy donors (2743, 2744, W070522016567, W070522116402, W070522116409, W070522002639, W070522002641 and W070522002642) and fixing and staining them by the same operator on the same day, and running on CyTOF on the next day. All steps were performed by the same operator. Donors were of unknown sex and age. Median, standard deviation and range was calculated for each major immune population and can be seen in Table 10. Reference ranges were established in healthy subjects for all but three populations(CD4 TEMRA, plasmablasts and transitional DCs). These are rare cell populations that in healthy individuals are present at low frequencies. Burel et al. (2017) found CD4 TEMRA cells present in healthy PBMCs at a range of 0.27% - 17.9%, with an average of 2.79% and a standard deviation of 4.41%, but they also reported a high CV for their validation analyses. They determined CD4 TEMRA were poorly resolved -

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meaning the markers that define them are dimly expressed, or there is no clear separation between positive and negative populations. Mei found that plasmablasts are present at only 0.14% of PBMCs in healthy patients, with a standard deviation of 39% and a range of 0.03%-2.39%. Frequencies were much higher in patients who received a recent vaccination or infection. Finally, transitional DCs are a subset of plasmacytoid DCs and have only been recently defined. We could not find a reference frequency in other literature, but they are present at <0.5% of PBMCs and play a role in viral infections, so they are likely present at very low frequencies in healthy subjects (Alcántara-Hernández et al. 2017). As such, these populations are likely present at higher levels in patients with an infection or other illness, but not in healthy subjects, which makes establishing a reference range in healthy people challenging. As such, we deemed it acceptable to not have reference values in healthy patients for these populations.

Table 10: Reference Range Values for PBMCs from Healthy Subjects

Cell Subset	# Subjects	% of non-granulocytes		
		Median	Standard deviation	Range
T cells	8	37.28	17.41	4.28 - 51.69
CD4+ T cells	8	16.06	12.30	2.29 - 36.69
Naive	8	4.09	7.33	0.26 - 19.64
TFH	7	0.31	0.21	0.09 - 0.73
Treg	8	1.05	0.89	0.19 - 2.81
TCM	8	7.35	4.63	1.50 - 13.95
TEM	8	1.02	1.85	0.18 - 5.47
TEMRA	2*	*	*	*
CD8+ T cells	8	9.33	4.58	0.98 - 13.65
Naive	8	1.84	2.27	0.28 - 6.68
TCM	8	2.27	1.19	0.42 - 3.33
TEM	6	2.29	2.63	0.13 - 5.53
TEMRA	6	1.88	2.53	0.12 - 5.70
γδ T cells	8	1.58	1.47	0.27 - 4.01
NKT cells	7	1.07	3.37	0.25 - 9.62
DNT	8	0.95	0.78	0.33 - 2.40

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DPT	5	0.12	0.06	0.10 - 0.25
B cells	8	6.78	4.69	4.06 - 17.30
Naive	8	5.35	3.23	2.58 - 13.02
Memory	8	1.57	2.24	1.02 - 7.51
PB	0*	*	*	*
Dendritic Cells	8	2.55	1.27	1.52 - 5.53
cDC	8	1.57	0.75	0.71 - 3.12
pDC	8	0.47	0.49	0.15 - 1.69
tDC	3*	*	*	*
Monocytes	8	26.26	17.73	14.75 - 57.98
cMono	8	22.46	15.70	12.07 - 53.84
inMono	8	0.47	0.33	0.26 - 1.13
ncMono	8	3.02	3.26	1.80 - 11.03
NK cells	8	17.86	9.50	7.60 - 35.63
CD16+	8	16.90	9.54	6.72 - 34.80
CD16-	8	0.53	0.38	0.25 - 1.33
CD56+	8	0.42	0.20	0.24 - 0.83

**Populations with fewer than 100 cell events were excluded from analysis. Populations in which fewer than 5 replicates had sufficient cell count for analysis were excluded from reference range calculations. As a result, we excluded CD4+ TEMRA cells, Plasmablasts and Transitional Dendritic Cells from reference range calculations.*

Data for individual donors can be found in DCS122 Appendix B Teiko Bio Pan-Immune Profiling Test Validation Report Data.

8.6 Summary

Teiko Bio's Pan-Immune Profiling assay was built on a panel with optimal signal for each marker and minimal spillover into other channels. The assay was able to detect all major immune population subsets within T cells, B cells, NK cells, monocytes and dendritic cells in PBMCs and was used to set a reference range for nearly all populations in PBMCs from healthy subjects. The acceptance criteria for precision within runs (intra-run), between runs (inter-run) and between operators (inter-operator) were met for all reportable cell populations. In addition, the acceptance criteria for stability up to 21 days were met for all reportable cell populations for cryopreserved PBMC samples stored at -80C.

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9. References

Appendices

- [DCS122 Appendix A Teiko Pan-Immune Profiling Panel Antibody Titration Data](#)
- [DCS122 Appendix B Teiko Bio Pan-Immune Profiling Test Validation Report Data](#)

Standard Operating Procedures

- [DCS003 PBMC fixation with PFA](#)
- [DCS006 Preparation of CyTOF reagents](#)
- [DCS007 CyTOF Staining with Barcode](#)
- [DCS020 Isolation of PBMC by Ficoll](#)
- [DCS034 Quality Assurance and Performance Verification](#)
- [DCS086 Performance Validation Plan](#)

White Papers

- [Teiko Bio's Panel and Custom Antibody Validation Process](#) - White Paper, last updated March 2023

Research Articles

- Alcántara-Hernández et al. [High-dimensional phenotypic mapping of human dendritic cells reveals interindividual variation and tissue specialization](#). *Immunity* (2017). 47, 1037-1050
- Burel et al. [An integrated workflow to assess technical and biological variability of cell population frequencies in human peripheral blood by flow cytometry](#). *The Journal of Immunology* (2017). 198 (4): 1748 - 1758
- Mei et al. [Blood-borne human plasma cells in steady state are derived from mucosal immune responses](#). *Blood* (2009). 113 (11): 2461-2469

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10. History block

Revision	Originator	Date Effective	Nature of Change
01	Jolien Sweere	06/20/2023	Initial release
02	Jolien Sweere	10/17/2023	Rename test to Pan-Immune Profiling Test

Reviewed by

Name	Date	Signature
Jacek Klepacki	07/22/2023	
Li-Chun Cheng	2023.7.20	