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Method Validation Report for A Mass Cytometry Assay to Determine the Pan-Immune Profile of Whole Blood Collected Using the Sodium Heparin Stable-Lyse Stable-Store TokuKit

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Teiko Bio, Inc.

675 Arapeen Dr, Suite 301

Salt Lake City, UT, 84108

USA

1. Table of Content

2. Compliance Statement

The validation experiments were performed as per the validation study plan described in DCS130 CyTOF TokuKit SH 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 whole blood, including subsets in T cells, B cells, NK cells, monocytes and dendritic cells using mass cytometry (CyTOF). Whole blood is prepared for CyTOF processing using Teiko's TokuKit, which uses sodium heparin as an anticoagulant and Stable-Lyse, Stable-Store (SmartTube Inc) as cell fixative. 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 whole blood collected from healthy humans using Teiko's Sodium Heparin, Stable-Lyse Stable-Store TokuKit, hereby referred to as TokuKit SH. The method follows the general development and validation procedure described in DCS130 CyTOF TokuKit SH 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 document describes the following performance validation results:

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

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

5. Responsibility

The Laboratory Director of the laboratory and the Technical 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
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
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
TokuKit SH	Teiko's blood collection kit using sodium heparin anticoagulant and Stable-Lyse, Stable-Store as fixative

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 granulocytes, T cells, B cells, NK cells and myeloid cells in whole blood prepared for mass cytometry with Teiko's TokuKit SH. The TokuKit SH uses sodium heparin as an anticoagulant and Stable-Lyse, Stable-Store (SmartTube Inc) as cell fixative.

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 granulocytes, T cells, B cells, NK cells, and myeloid cells. 41 antibodies are used to stain whole blood from healthy human donors collected with Teiko's TokuKit SH. In this report, the performance of the panel is described for different validation parameters: accuracy, precision, 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
Granulocyte subsets will be measured as % of total leukocytes. Leukocytes are defined as live CD61- CD235a- singlets and eosinophils. Other cell lineages will be measured as % of total non-granulocytes.

Cell Subset	Definition (Marker Parameters)
Granulocytes (gating starts from Live Singlets)	
Eosinophils	Siglec8+ CD66b+
Basophils	CD61- CD235a- CD123+ HLA-DR- non-eosinophils
Mast Cells	cKit+ FcER1+ non-basophils
Neutrophils	CD66b+ non-mast cells
Non-Granulocytes (gating starts from CD45+ non-neutrophils)	
B cells	CD19+ CD3- CD14- CD33-
Naive	CD19+ CD3- CD14- CD33- CD38+ CD27-
Memory	CD19+ CD3- CD14- CD33- CD38-
Plasmablasts (PB)	CD19+ CD3- CD14- CD33- CD38hi CD27+
T cells	CD3+ CD19- CD14- CD33-
Gamma Delta (γδ) T cells	CD3+ CD19- CD14- CD33- gdTCR+
Natural Killer T cells (NKT)	CD3+ CD19- CD14- CD33- gdTCR- CD56+
CD4/CD8 Double-Negative T	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8-

cells (DNT)	
CD4/CD8 Double-Positive T cells (DPT)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8+
CD4+ T cells	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8-
Regulatory T cells (Treg)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD127lo CD25+ FOXP3+
Naive	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD25- CD45RA+ CD27+
Central Memory (TCM)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD25- CD45RA- CD27+
Effector Memory (TEM)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD25- CD45RA- CD27-
CD45RA+ Effector Memory (TEMRA)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD25- CD45RA+ CD27-
CD8+ T cells	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+
Naive	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+ CD25- CD45RA+ CD27+
Central Memory (TCM)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+ CD25- CD45RA- CD27+
Effector Memory (TEM)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+ CD25- CD45RA- CD27-
CD45RA+ Effector Memory (TEMRA)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+ CD25- CD45RA+ CD27-
Natural Killer (NK) cells	CD3- CD19- CD14- CD33- CD56+ CD7+
CD16+	CD3- CD19- CD14- CD33- CD7+ CD56+ CD16+
CD16-	CD3- CD19- CD14- CD33- CD7+ CD56+ CD16-
CD56+	CD3- CD19- CD14- CD33- CD7+ CD56hi CD16-
Dendritic Cells	HLA-DR- CD14- CD16- CD123+ OR CD11c+ non-NK cells
Classical (cDC)	HLA-DR- CD14- CD16- CD123- CD11c+ non-NK cells
Plasmacytoid (pDC)	HLA-DR- CD14- CD16- CD123+ CD11c- non-NK cells
Transitional (tDC)	HLA-DR- CD14- CD16- CD123+ CD11c+ non-NK cells
Monocytes	HLA-DR+ CD14+ OR CD16+ non-NK cells
Classical (cMono)	HLA-DR+ CD14+ CD16- non-NK cells
Intermediate (inMono)	HLA-DR+ CD14+ CD16+ non-NK cells
Non-Classical (ncMono)	HLA-DR+ CD14- CD16+ non-NK cells
Monocyte myeloid-derived suppressor cells (MoMDSCs)	HLA-DR- CD14+ CD33+ non-NK cells

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
   a. Helios mass cytometer #1, by Standard BioTools, serial number 1070010393C
   b. Helios mass cytometer #2, by Standard BioTools, serial number 1070011428C
2. Software
   a. CellEngine, version January 2024 (CellCarta, Montreal, Canada)
   b. CyTOF Software v7.0.8493 (Standard BioTools, South San Francisco, California, USA)
   c. RStudio Desktop 2023.06.1+524 (Posit, Boston, Massachusetts, USA)
   d. Premessa R script 0.3.4 on GitHub (San Francisco, California, USA)
   e. ggplot2 R package version: 3.4.2 on GitHub (San Francisco, California, USA)
3. Method
   Whole blood from healthy donors was processed using Teiko's TokuKit SH, which uses sodium heparin as anticoagulant and Stable-Lyse, Stable-Store (SmartTube Inc) as preservative. Processed whole blood was stained with Teiko Bio's Pan-Immune Profiling Test consisting of metal-conjugated antibodies. Major immune cell populations such as neutrophils, T, B, NK and myeloid cells and their corresponding subpopulations were evaluated based on the combination of metal signals measured by mass cytometer.
4. Reagents

Table 2: List of critical reagents to be used for validation

Material	Manufacturer	Catalog #
Stable-Lyse V2	SmartTube Inc	501351694
Stable-Store V2	SmartTube Inc	501351693
Sodium heparin vacutainers	BD Biosciences	367871
Nalgene bottles	Nalgene	3220029050
Transfer pipettes	Falcon	357524
Trypan Blue Dye 0.4% [2X stock]	Thermo Scientific	T10282
Countess™ Cell Counting Slide Chamber	Thermo Scientific	C10228
1X phosphate-buffered saline	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
Cell staining buffer	Made in-house, see DCS006	n/a
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

0.5M EDTA, pH 8.0 (100X)	Thermo Fisher	15575020
30% BSA	Sigma Aldrich	A728450ML
EQ Four Element Calibration Beads	Standard BioTools	201078
Dimethylsulfoxide (DMSO)	Sigma	D2650

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	MAB098	5/1/2024
111	Cd	CD61	VI-PL2	Biolegend	336402	MAB287	5/1/2024
111	CS	CD235ab	HIR2	Biolegend	306602	MAB286	5/1/2024
112	Cd	CD8	SK1	Biolegend	344702	MAB315	5/1/2024
113	In	CD33	WM53	Biolegend	303438	MAB177	5/1/2024
114	Cd	HLA-DR	L243	Biolegend	307602	MAB109	5/1/2024
115	In	CD66b	G10F5	Biolegend	305102	MAB099	5/1/2024
116	Cd	CD11b	ICRF44	Biolegend	301302	MAB110	5/1/2024
141	Pr	CD3	UCHT1	Biolegend	300438	MAB173	5/1/2024
142	Nd	CD19	HIB19	Biolegend	302202	MAB127	5/1/2024
143	Nd	cKit	104D2	Biolegend	313202	MAB305	5/1/2024
144	Nd	CD15	W6D3	Biolegend	323002	MAB304	5/1/2024
145	Nd	CD4	RPA-T4	Biolegend	300570	MAB114	5/1/2024
146	Nd	FcER1	AER-37	Biolegend	334648	MAB301	5/1/2024
147	Sm	CD11c	Bu15	Biolegend	337202	MAB063	5/9/2024
148	Nd	CD14	M5E2	Biolegend	301862	MAB128	5/14/2024
149	Sm	CD7	M-T701	BD	555359	MAB048	5/1/2024
150	Nd	CD86	IT2.2	Biolegend	305402	MAB116	5/9/2024
151	Eu	CD123	6H6	Biolegend	306002	MAB066	4/30/2024
152	Sm	gdTCR	B1	Biolegend	331202	MAB067	5/1/2024
153	Eu	CD45RA	HI100	Biolegend	304102	MAB077	5/1/2024
154	Sm	TIM3	F38-2E2	Biolegend	345002	MAB070	5/1/2024
156	Gd	PD-L1	29E.2A3	Biolegend	329702	MAB071	5/1/2024
157	Gd	Siglec-8	7C9	Biolegend	347102	MAB306	5/1/2024
158	Gd	CD27	L128	Standard BioTools	3158010B	MAB016	5/1/2024
159	Tb	TCF1	C63D9	Cell Signaling	2203BF	MAB328	11/7/2024
160	Gd	Tbet	4B10	Biolegend	644802	MAB080	5/1/2024
161	Dy	CTLA-4	14D3	Standard BioTools	3161004B	MAB319	8/24/2024
162	Dy	Foxp3	PCH101	Standard BioTools	3162011A	MAB019	5/1/2024
164	Dy	CD161	HP-3G10	Biolegend	339902	MAB082	5/1/2024
165	Ho	CD127	R34-34	Novus Biologicals	DDX0700P100	MAB170	5/1/2024

166	Er	CD74	LN2	Biolegend	326802	MAB085	5/1/2024
167	Er	CCR7	G043H7	Standard BioTools	3167009A	MAB316	5/1/2024
168	Er	Ki67	Ki-67	Biolegend	350502	MAB087	5/1/2024
169	Tm	CD25	M-A251	Biolegend	356102	MAB045	5/1/2024
170	Er	LOX-1	15C4	Biolegend	358602	MAB074	5/1/2024
171	Yb	TIGIT	MBSA43	Thermo Fisher	16-9500-82	MAB035	5/1/2024
172	Yb	CD38	HIT2	Biolegend	303502	MAB089	4/30/2024
174	Yb	PD-1	EH12.2H7	Biolegend	329902	MAB122	5/1/2024
175	Lu	LAG-3	11C3C65	Biolegend	369302	MAB076	5/1/2024
176	Yb	CD56	NCAM16.2	BD	559043	MAB090	5/1/2024
209	Bi	CD16	3G8	Biolegend	302050	MAB269	5/1/2024

All experiments were completed before any antibodies expired.

5. Sample Processing

a. **Sample collection**

Whole blood from 3 donors (subject IDs SBC-1, SBC-2 and SBC-3) were purchased from the Stanford Blood Bank (Palo Alto, CA, USA) collected in sodium heparin vacutainers. Whole blood from another 3 donors (subject IDs D1H, D2H and D3H) were purchased from ARUP Laboratories (Salt Lake City, UT, USA) as collected in sodium heparin vacutainers. Whole blood from the Stanford donors were used in the majority of the described experiments. Blood from these donors was processed within 2 hours of collection using the Teiko TokuKit with Stable-Lyse, Stable-Store cell preservative according to the manual, and shipped overnight on dry ice to Teiko's laboratory in Salt Lake City, UT. There, samples were stored at -80C until use. Blood from the ARUP donors were used for the pre- and post-fixation stability experiments and were processed as described in the stability section below.

b. 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 DCS007B CyTOF Staining with Barcode. Briefly, after counting, 1 million cells were washed twice with eBioscience perm buffer and incubated for 15 minutes in the perm buffer during the second wash. Cells were washed twice with PBS and then barcoded with unique combinations of Pd isotopes for 30 min at room temperature on a shaker in PBS. After barcoding, cells were washed twice with Cell Staining Buffer (CSB) and pooled into a single tube.

c. Antibody staining

Antibody staining was performed as described in DCS007B 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 µL 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 room temperature. PBMC were stained using a panel of 42 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 supplemented with 10% DMSO and frozen at -80C until the day of acquisition.

d. **Data acquisition**

As described in DCS007B 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).

e. **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 most precision analyses, each population as a percentage of live cells or 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 * standard deviation(frequency) / mean(frequency). For inter-instrument precision, we calculated the percent change between machines as follows: % change = 100 * (frequency on Helios #2 - frequency on Helios #1) / frequency on Helios #1. The precision assessment data were visualized with R using the ggplot2 package to display the %CV vs. the mean cell count of the population.

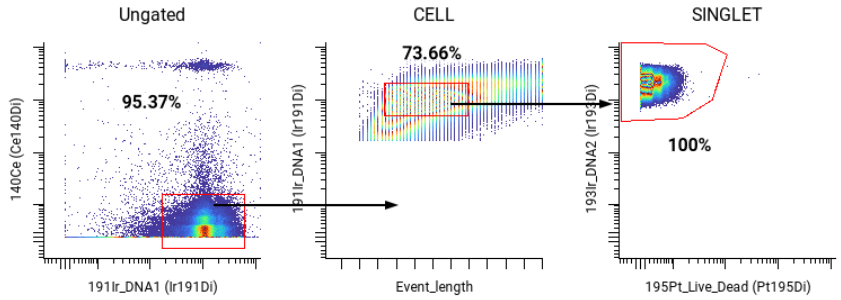
For stability assessments, the percent change between timepoints was calculated as follows: % change = 100 * (frequency at t=*n* - frequency at t=0) / frequency at t=0. The data were visualized with R using the ggplot2 package to display the median percent change between t=0 and subsequent timepoints, 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(percent change between t=0 and t=*n*)/sqrt(number of samples).

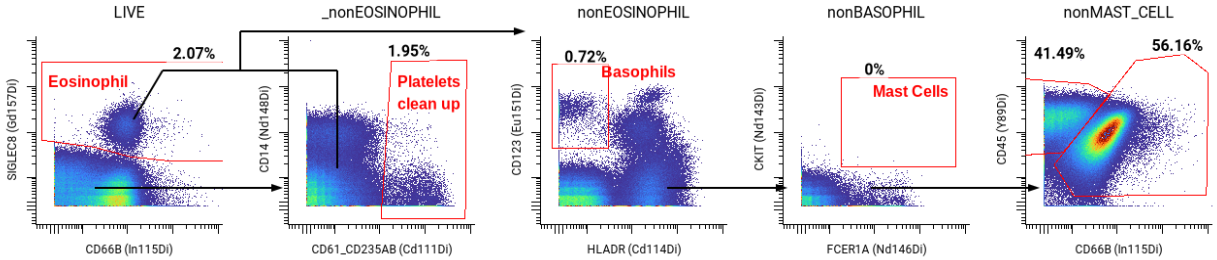
For the reference range experiment, we calculated the median, standard deviation, minimum, and maximum percent of frequency for each population.

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

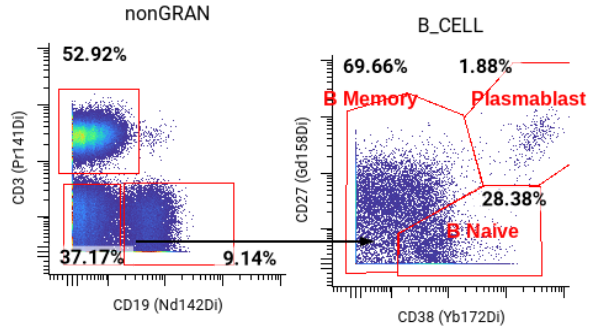
A. Gating strategy for live cells (from total events)



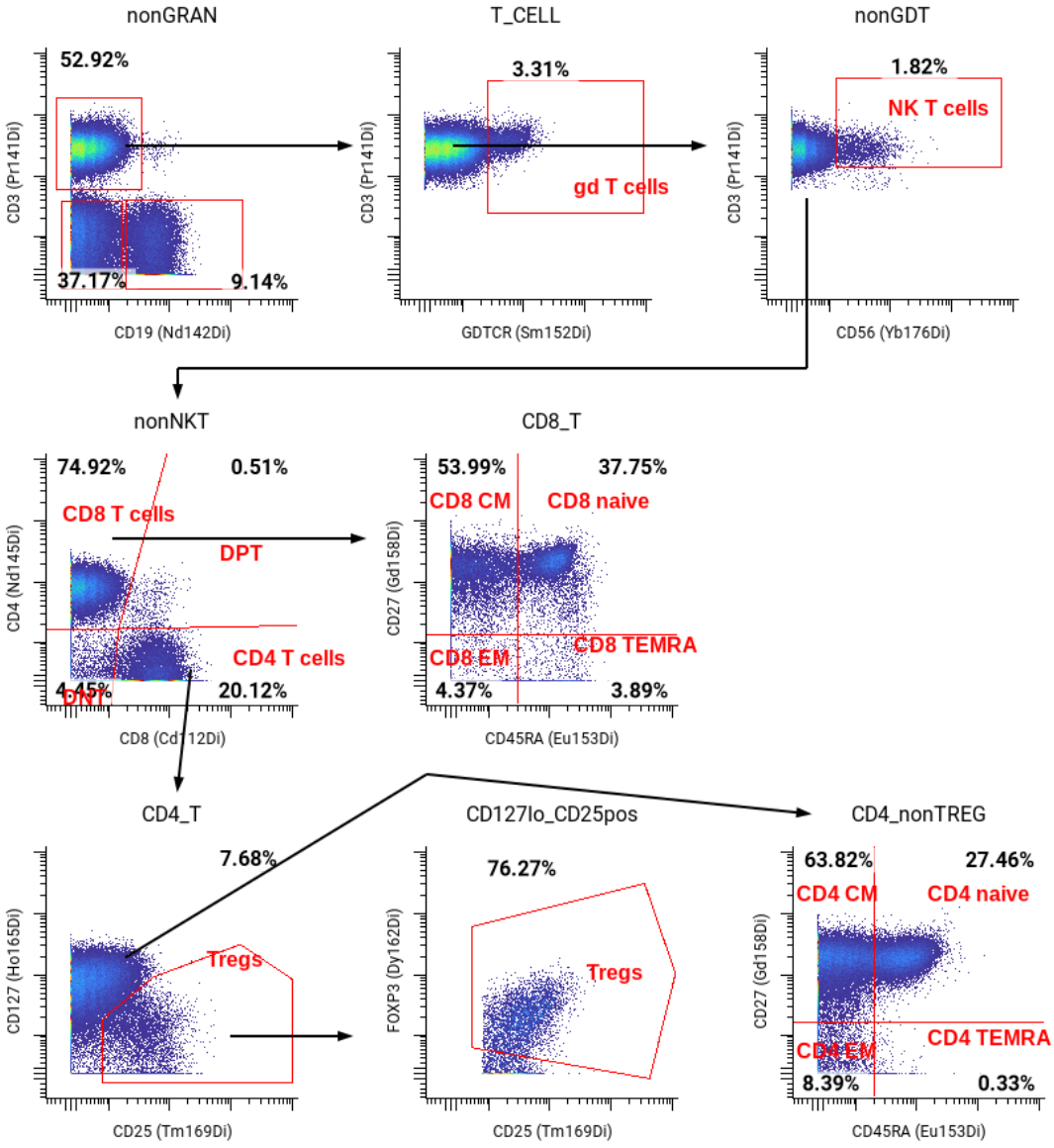
B. Gating strategy for granulocytes (from live cells)



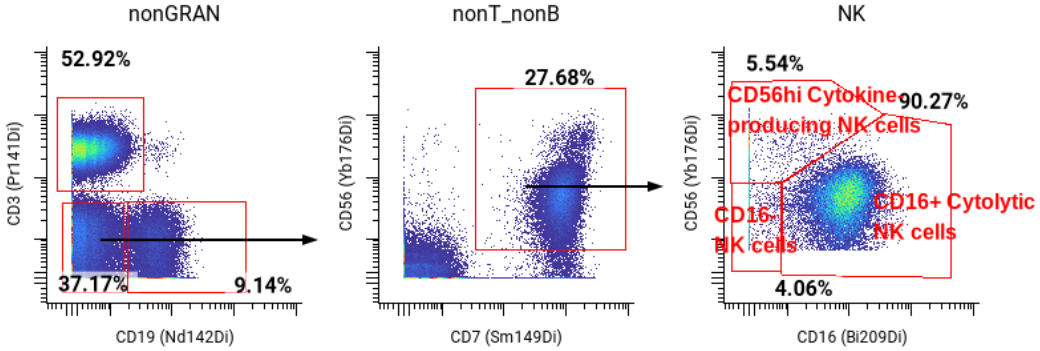
C. Gating strategy for B cells (from non-granulocytes)



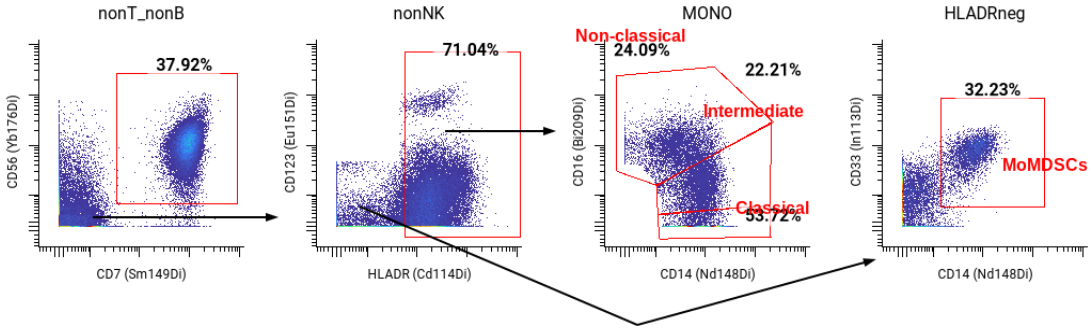
D. Gating strategy for T cells (from non-granulocytes)



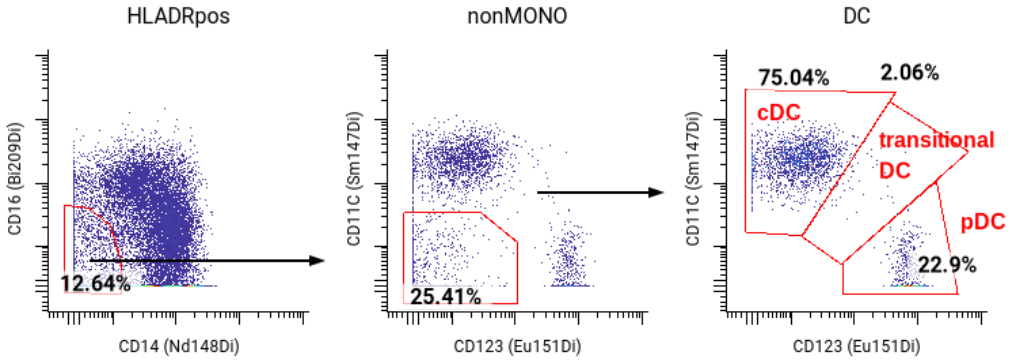
E. Gating strategy for NK cells (from non-granulocytes)



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



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



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
   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 whole blood from 6 healthy subjects (SBC-1, SBC-2, SBC-3, D1H, D2H and D3H).

2. Precision
   Intra-run, Inter-run, Inter-instrument and Inter-kit precision was evaluated to ensure test results are not affected by technical intra-run or inter-run variables, or by operator variables during the whole blood processing step, or by the individual mass cytometry instrument the samples are run on.
   a. Intra-run precision was assessed by taking whole blood from three (3) healthy donors (SBC-1, SBC-2 and SBC-3) and preserving each blood sample with TokuKit-SH. After preservation, each blood sample was split into three technical replicates, and all replicates were stained and run by the same operator, who ran all samples together on the same CyTOF machine during one run. Variance between sets of replicates was calculated for precision analysis. Acceptance criteria were ≥95% of immune measurements should have a CV ≤ 25% between replicates.
   b. Inter-run precision was assessed by taking whole blood from three (3) healthy donors (SBC-1, SBC-2 and SBC-3) and preserving each blood sample with TokuKit-SH. After preservation, each blood sample was split into three technical replicates. Each set of replicates was stained and run on three separate days. One operator performed all steps. Variance between days was calculated for precision analysis. Acceptance criteria were ≥95% of immune measurements should have ≤30% CV between individual runs.
   c. Inter-instrument precision was assessed by taking whole blood from three (3) healthy donors (SBC-1, SBC-2 and SBC-3) and preserving each blood sample with TokuKit-SH. After preservation, each blood sample was split into two technical replicates. All replicates were processed together in the same manner by the same operator. One replicate was run on the Helios #1, and the other was run on the Helios #2. Change in cell lineage frequency between Helios #2 and Helios #1 was calculated for precision analysis. Acceptance criteria were ≥95% of immune measurements should have ≤25% change in frequency between individual instruments.
   d. Inter-kit precision was assessed by taking whole blood from three (3) healthy donors (SBC-1, SBC-2 and SBC-3) and splitting the blood from each donor *before* TokuKit processing into three replicates. Three different operators fixed one replicate from each

donor in an individual TokuKit, for 3 kits per donor and 9 samples total. One (1) operator subsequently stained and ran all samples on the same CyTOF. Variance between TokuKit-processed replicates within each donor was calculated for precision analysis. Acceptance criteria were ≥95% of immune measurements should have a CV ≤ 25% between donor-matched TokuKits.

3. Stability

   Stability was evaluated to ensure test results are not affected by time until fixation or storage-related variables.

   a. Pre-fixation stability was assessed by taking whole blood collected in sodium heparin vacutainers from 3 (three) healthy donors (D1H, D2H and D3H). The vacutainers, containing donor blood, were stored at room temperature. 2 mL of blood from each donor was processed using the TokuKit within three hours of collection (t=0), and then at regular intervals after the initial processing time point: t=2 hours, t=4 hours, t=6 hours, t=24 hours and t=48 hours, for a total of 6 timepoints. All samples were processed, stained and run on the Helios #1 by the same operator. The percentage of change in immune population frequencies for each time point compared to t=0 was calculated. Acceptance criteria were ≥95% of immune feature measurements at each time point should have ≤ 25% change in immune population frequencies when compared to t=0.

   b. Post-collection stability was assessed by taking whole blood from 3 (three) healthy donors (D1H, D2H, and D3H) and processing them with Teiko's TokuKit. All samples were split into 6 technical replicates and stored at -80C on 11-07-2023. The first set of replicates was thawed the next day, 11-08-2023, and run on CyTOF that same day, t=0. Another set of replicates was thawed and run on CyTOF on 12-08-2023, t=1 month. All steps were performed by the same operator. Percentage of change in immune population frequencies between months was calculated for stability analysis. Acceptance criteria were ≤25% change in immune population frequencies between t=0 and t=1 month. The remainder of the replicates will be tested at t=3 months, t=6 months, t=9 months and t=12 months, but are not included in this version of the report.

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 whole blood from 6 (six) healthy donors (SBC-1, SBC-2, SBC-3, D1H, D2H and D3H) and processing them for CyTOF. 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.

8.5 Results

1. 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

Channel	Metal	Protein	Clone	Manufacturer	Optimal Concentration (µg/mL)	Stock Concentration (mg/mL)	Volume per 100 µL test
89	Y	CD45	HI30	Biolegend	1.5	0.3	0.5
111	Cd	CD61	VI-PL2	Biolegend	0.1875	0.075	0.25
111	CS	CD235ab	HIR2	Biolegend	0.1875	0.075	0.25
112	Cd	CD8	SK1	Biolegend	0.75	0.075	1
113	In	CD33	WM53	Biolegend	1.5	0.3	0.5
114	Cd	HLA-DR	L243	Biolegend	1.5	0.3	0.5
115	In	CD66b	G10F5	Biolegend	0.75	0.3	0.25
116	Cd	CD11b	ICRF44	Biolegend	3	0.3	1
141	Pr	CD3	UCHT1	Biolegend	0.75	0.3	0.25
142	Nd	CD19	HIB19	Biolegend	0.75	0.3	0.25
143	Nd	cKit	104D2	Biolegend	0.1875	0.3	0.0625
144	Nd	CD15	W6D3	Biolegend	6	0.3	2
145	Nd	CD4	RPA-T4	Biolegend	0.375	0.15	0.25
146	Nd	FcER1	AER-37	Biolegend	0.1875	0.075	0.25
147	Sm	CD11c	Bu15	Standard BioTools	3	0.3	1
148	Nd	CD14	M5E2	Biolegend	0.75	0.3	0.25
149	Sm	CD7	M-T701	BD	3	0.3	1
150	Nd	CD86	IT2.2	Biolegend	0.75	0.3	0.25
151	Eu	CD123	6H6	Standard BioTools	NA	0.3	1
152	Sm	gdTCR	B1	Biolegend	3	0.3	1
153	Eu	CD45RA	HI100	Biolegend	0.75	0.075	1
154	Sm	TIM3	F38-2E2	Biolegend	3	0.3	1
156	Gd	PD-L1	29E.2A3	Biolegend	1.5	0.15	1
157	Gd	Siglec-8	7C9	Biolegend	6	0.3	2
158	Gd	CD27	L128	Standard BioTools	NA	0.3	0.25
159	Tb	TCF1	C63D9	Cell Signaling	0.375	0.075	0.5
160	Gd	Tbet	4B10	Biolegend	0.75	0.3	0.25

161	Dy	CTLA-4	14D3	Thermo Fisher	0.75	0.3	0.25
162	Dy	Foxp3	PCH101	Standard BioTools	NA	0.3	0.5
164	Dy	CD161	HP-3G10	Biolegend	3	0.3	1
165	Ho	CD127	R34-34	Novus Biologicals	1.5	0.075	2
166	Er	CD74	LN2	Biolegend	0.375	0.15	0.25
167	Er	CCR7	G043H7	Standard BioTools	NA	0.3	0.25
168	Er	Ki67	Ki-67	Biolegend	0.75	0.3	0.25
169	Tm	CD25	M-A251	Biolegend	1.5	0.3	0.5
170	Er	LOX-1	15C4	Biolegend	0.1875	0.075	0.25
171	Yb	TIGIT	MBSA43	Thermo Fisher	3	0.6	0.5
172	Yb	CD38	HIT2	Standard BioTools	1.5	0.2	0.75
174	Yb	PD-1	EH12.2H7	Biolegend	0.75	0.075	1
175	Lu	LAG-3	11C3C65	Biolegend	0.375	0.15	0.25
176	Yb	CD56	NCAM16.2	BD	0.1875	0.075	0.25
209	Bi	CD16	3G8	Biolegend	1.5	0.3	0.5

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 whole blood isolated from 6 healthy subjects (SBC-1, SBC-2, SBC-3, D1H, D2H and D3H) to establish the test's ability to detect all individual major immune cell populations from these mixed samples. We were able to detect nearly all major immune cell populations and subsets within granulocytes, T cells, B cells, NK cells, monocytes and dendritic cells. Figures 2-7 show data averaged across the 6 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 DCS131 Appendix A CyTOF TokuKit SH Performance Validation Report Data. Populations for which none of the subjects had sufficient cells (sufficient events to pass limit of detection) included mast cells and transitional DCs.

Figure 2: Median frequency of major immune lineages in whole blood of healthy subjects across all 6 donors.

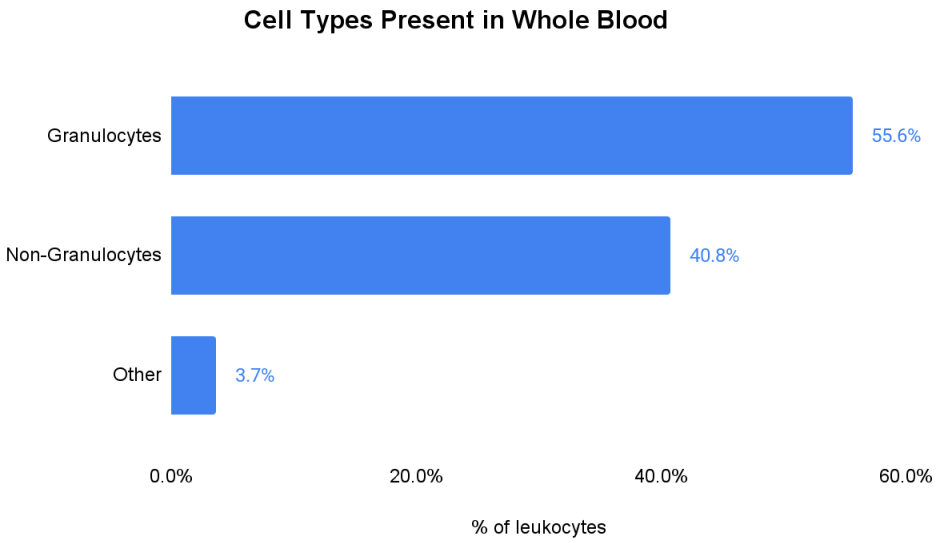


Figure 3: Median frequency of major immune cell types in granulocytes of healthy subjects across all 6 donors.

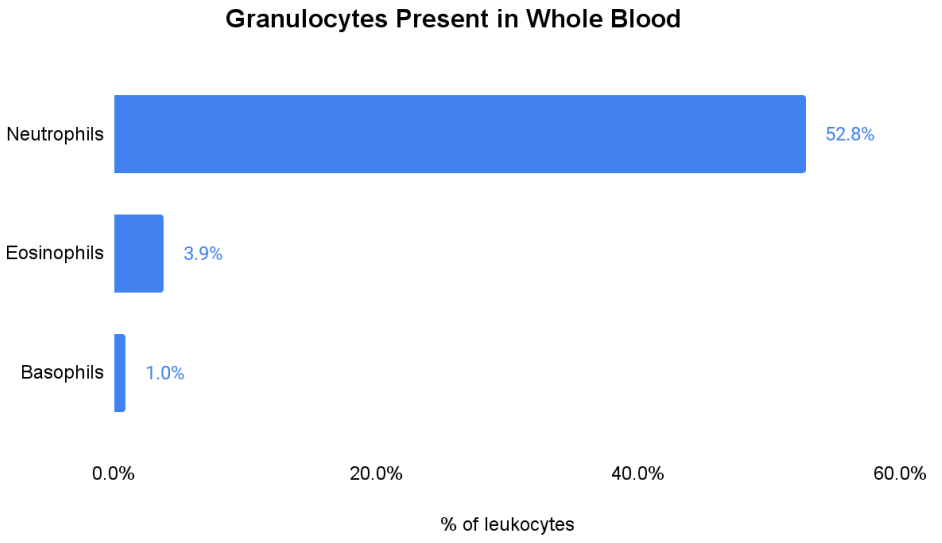
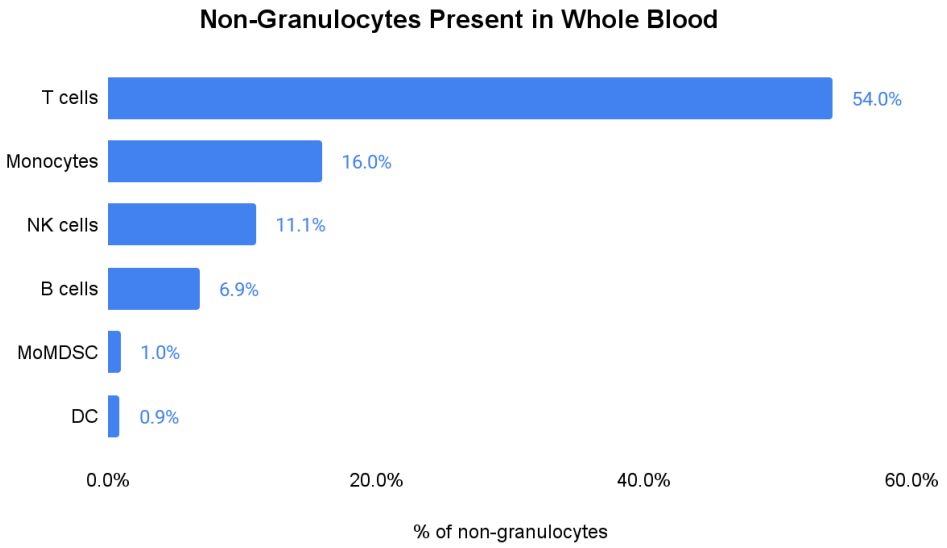
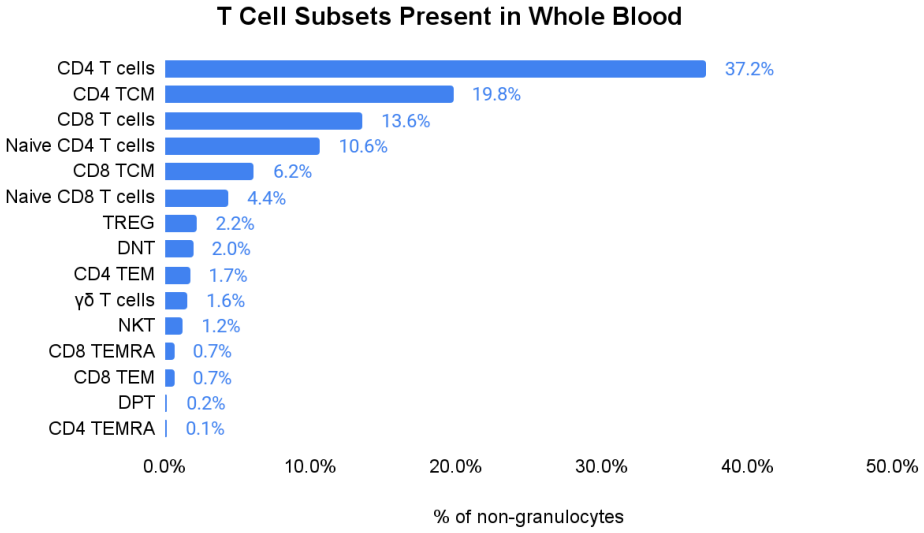


Figure 4: Median frequency of major immune cell types in non-granulocytes of healthy subjects across all 6 donors.



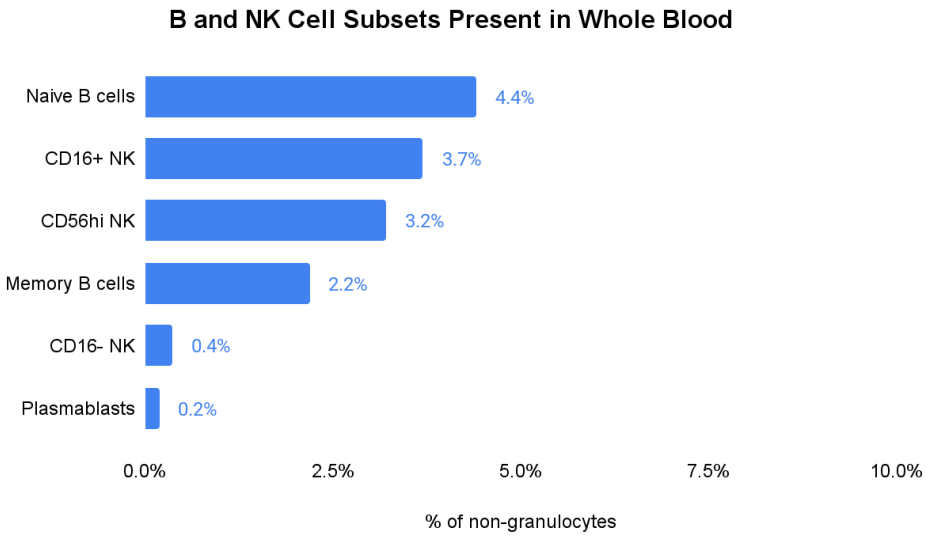
Note that MoMDSC has only n=3 donor samples due to populations < 100 events.

Figure 5: Median of individual T cell subset frequencies of healthy subjects across all 6 donors.



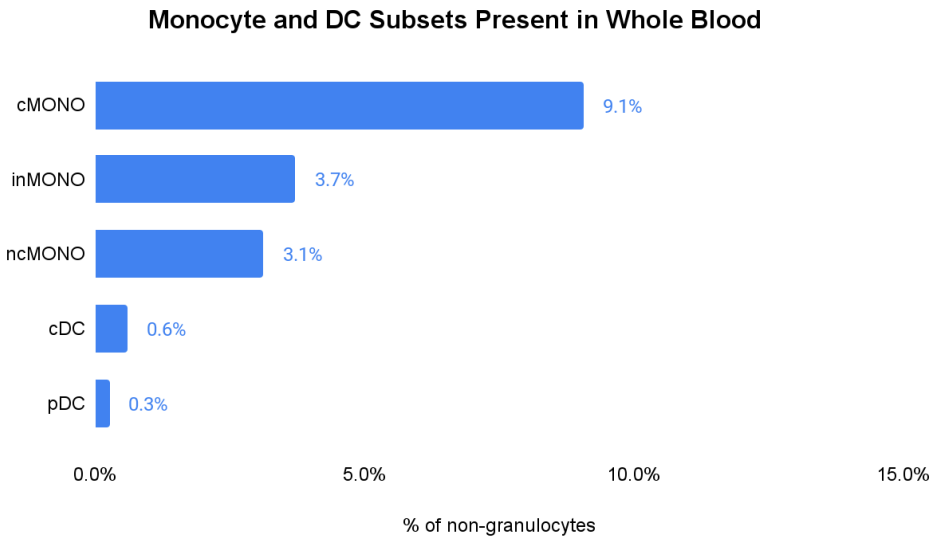
Note that CD4 TEMRA has only n=2 donor samples due to populations < 100 events.

Figure 6: Median of individual B and NK cell subset frequencies of healthy subjects across all 6 donors.



Note that plasmablasts have only n=2 donor samples due to populations < 100 events.

Figure 7: Median of individual monocyte (mono) and dendritic cell (DC) subset frequencies of healthy subjects across all 6 donors.



2. Precision
   a. Intra-Run

   Intra-run precision was assessed by taking whole blood from three (3) healthy donors (SBC-1, SBC-2 and SBC-3), splitting each 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 Tables 5-7 and Figure 8. All measurable (>100 cells median) cell populations passed the acceptable threshold of ≤25% variation between replicates. **Total average intra-run %CV for all measurable populations across all three donors was 2.16%**, with individual donor average %CVs of 2.43%, 1.47%, and 2.56% for Donor 1 (SBC-1), Donor 2 (SBC-2), and Donor 3 (SBC-3), respectively.

Table 5: Summary of Intra-Run Precision Assessment for Donor 1 (SBC-1)

Cell Subset	# Replicates	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (%)
Granulocytes				
Eosinophils	3	11327	2.39	8.52
Basophils	3	3393	0.71	8.60
Mast Cells	0*	*	*	*
Neutrophils	3	267555	52.88	4.90
Non-Granulocytes				
B cells	3	19150	9.88	0.54
Naive	3	5301	2.75	0.19
Memory	3	13340	6.91	0.91
Plasmablasts (PB)	3	432	0.22	5.09
T cells	3	97317	50.73	0.60
Gamma Delta (γδ) T cells	3	3121	1.62	0.87
Natural Killer T cells (NKT)	3	1668	0.87	1.14
CD4/CD8 Double-Negative T cells (DNT)	3	3783	1.96	0.44
CD4/CD8 Double-Positive T cells (DPT)	3	373	0.20	8.82
CD4+ T cells	3	69467	36.19	0.76
Regulatory T	3	4638	2.41	1.27

cells (Treg)				
Naive	3	17775	9.27	0.69
Central Memory (TCM)	3	40535	21.12	1.09
Effector Memory (TEM)	3	4997	2.61	0.38
CD45RA+ Effector Memory (TEMRA)	3	202	0.10	2.12
CD8+ T cells	3	18967	9.90	0.98
Naive	3	7161	3.73	1.44
Central Memory (TCM)	3	10325	5.37	0.75
Effector Memory (TEM)	3	773	0.41	4.38
CD45RA+ Effector Memory (TEMRA)	3	724	0.38	2.92
Natural Killer (NK) cells	3	19026	10.00	1.88
CD16+	3	17430	9.20	2.11
CD16-	3	405	0.21	7.02
CD56+	3	1133	0.58	1.75
Dendritic Cells	3	1854	0.97	1.97
Classical (cDC)	3	1176	0.61	2.03
Plasmacytoid (pDC)	3	646	0.34	2.55
Transitional (tDC)	0*	*	*	*
Monocytes	3	37178	19.29	0.95
Classical (cMono)	3	18441	9.58	0.49
Intermediate (inMono)	3	6809	3.50	1.09
Non-Classical (ncMono)	3	11919	6.21	1.97
Monocyte myeloid-derived suppressor cells (MoMDSCs)	3	2468	1.28	3.95
Donor 1 Average				**2.43**

**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 mast cells and transitional dendritic cells (tDCs) from analysis.

Table 6: Summary of Intra-Run Precision Assessment for Donor 2 (SBC-2)

Cell Subset	# Replicates	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (%)
Granulocytes				
Eosinophils	3	8717	1.80	0.70
Basophils	3	1846	0.38	1.23
Mast Cells	0*	*	*	*
Neutrophils	3	267739	55.70	0.35
Non-Granulocytes				
B cells	3	10900	5.79	1.16
Naive	3	9071	4.81	0.72
Memory	3	1810	0.96	3.17
PB	0*	*	*	*
T cells	3	107216	56.98	0.05
γδ T cells	3	22140	11.89	0.91
NKT	3	4129	2.22	1.12
DNT	3	4935	2.60	0.68
DPT	3	370	0.20	2.82
CD4+ T cells	3	47272	25.03	0.39
Treg	3	2158	1.15	1.96
Naive	3	21552	11.37	0.69
TCM	3	20643	10.99	0.82
TEM	3	2143	1.14	1.02
TEMRA	0*	*	*	*
CD8+ T cells	3	28370	15.04	0.27
Naive	3	16234	8.56	1.28
TCM	3	8862	4.75	2.19
TEM	3	1429	0.76	1.78
TEMRA	3	1824	0.97	1.38
NK cells	3	22178	11.70	0.84
CD16+	3	12625	6.69	0.87
CD16-	3	8578	4.51	1.16
CD56+	3	769	0.40	2.84
Dendritic Cells	3	2381	1.27	2.22
cDC	3	1646	0.87	0.85
pDC	3	643	0.35	8.33

tDC	0*	*	*	*
Monocytes	3	29450	15.63	0.36
cMono	3	17927	9.57	0.94
inMono	3	6738	3.57	1.38
ncMono	3	4699	2.50	1.82
MoMDSCs	3	2350	1.28	2.33
Donor 2 Average				**1.47**

**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 mast cells, CD4 TEMRA cells, plasmablasts (PB) and tDCs from analysis.*

Table 7: Summary of Intra-Run Precision Assessment for Donor 3 (SBC-3)

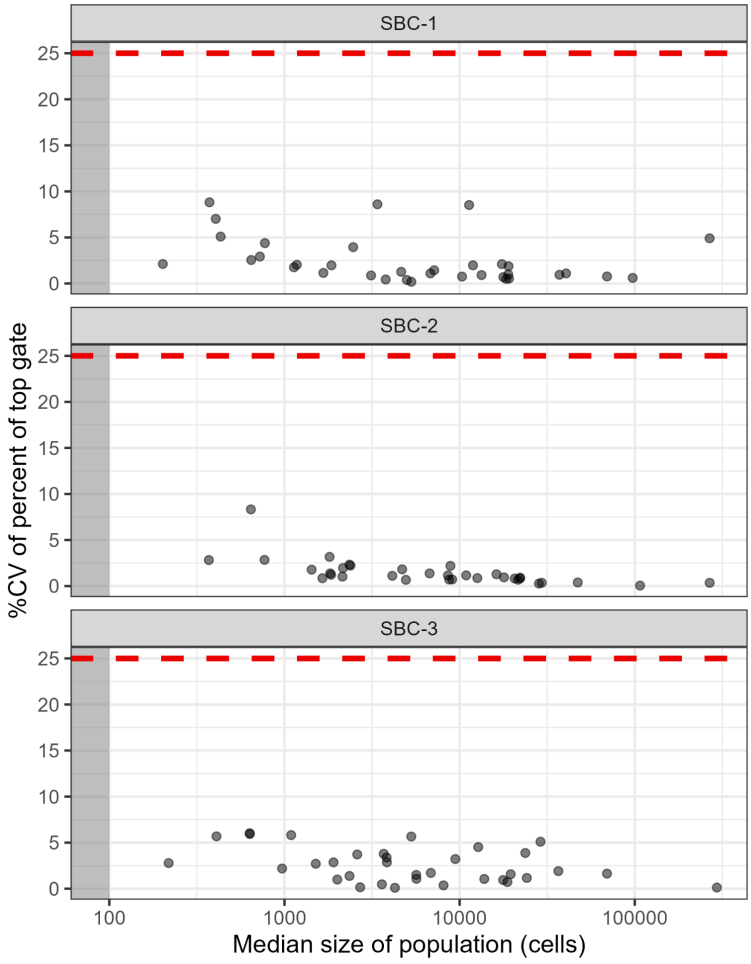
Cell Subset	# Replicates	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (%)
Granulocytes				
Eosinophils	3	28959	5.55	5.10
Basophils	3	5656	1.13	1.50
Mast Cells	0*	*	*	*
Neutrophils	3	294424	59.56	0.12
Non-Granulocytes				
B cells	3	8093	5.86	0.36
Naive	3	4273	3.09	0.10
Memory	3	3600	2.61	0.47
PB	3	218	0.16	2.78
T cells	3	69433	49.91	1.63
γδ T cells	3	3847	2.75	2.86
NKT	3	1092	0.77	5.82
DNT	3	2705	1.96	0.15
DPT	3	969	0.68	2.19
CD4+ T cells	3	36629	26.25	1.91
Treg	3	3686	2.63	3.79
Naive	3	9451	6.72	3.22
TCM	3	19574	14.05	1.57
TEM	3	2352	1.71	1.38
TEMRA	0*	*	*	*
CD8+ T cells	3	24191	17.49	1.16
Naive	3	6852	4.92	1.71
TCM	3	13827	10.06	1.06

TEM	3	2003	1.43	0.99
TEMRA	3	1509	1.08	2.70
NK cells	3	18737	13.61	0.72
CD16+	3	17728	12.89	0.94
CD16-	3	634	0.44	5.94
CD56+	3	409	0.28	5.68
Dendritic Cells	3	2602	1.84	3.72
cDC	3	1905	1.37	2.86
pDC	3	633	0.44	6.02
tDC	0*	*	*	*
Monocytes	3	23713	16.72	3.88
cMono	3	12766	8.95	4.52
inMono	3	5298	3.66	5.66
ncMono	3	5667	4.12	1.07
MoMDSCs	3	3829	2.71	3.39
Donor 3 Average				**2.56**

**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 mast cells, CD4 TEMRA cells and tDCs from analysis.*

Data for individual replicates can be found in DCS131 Appendix A CyTOF TokuKit SH Performance Validation Report Data.

Figure 8: Coefficient of Variation in Frequency (Percentage of Top Gate, which is Leukocytes or 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 whole blood processed with the TokuKit SH from three (3) healthy donors (SBC-1, SBC-2 and SBC-3) and splitting each sample into three technical replicates. Each set of replicates was stained and run on separate days. One operator performed all steps. Samples were stained and run on three separate days. Variance between runs was calculated for precision analysis and can be seen in Tables 8-10 and Figure 9. 97% of measurable (>100 cells median) cell populations passed the acceptable threshold of ≤30% variation between replicates, which were measured on different runs. **Total average interrun %CV for all measurable**

populations across all three donors was 6.83%, with individual donor average %CVs of 7.89%, 5.44%, and 7.09% for Donor 1 (SBC-1), Donor 2 (SBC-2), and Donor 3 (SBC-3), respectively. In donors SBC-1 and SBC-1, CD16- NK cells had %CV of ≥30%; total average %CV across all three donors is 28.74%.

Table 8: Summary of Inter-Run Precision Assessment for Donor 1 (SBC-1)

Cell Subset	# Replicates	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (%)
Granulocytes				
Eosinophils	3	9773	2.23	15.69
Basophils	3	3246	0.71	10.26
Mast Cells	0*	*	*	*
Neutrophils	3	253012	53.27	5.54
Non-Granulocytes				
B cells	3	17490	9.43	6.15
Naive	3	4953	2.66	5.61
Memory	3	12227	6.58	6.19
PB	3	308	0.19	21.95
T cells	3	93904	50.65	0.84
γδ T cells	3	3110	1.58	6.18
NKT	3	1655	0.89	0.60
DNT	3	3809	1.96	8.36
DPT	3	373	0.20	17.76
CD4+ T cells	3	66782	36.14	1.08
Treg	3	4081	2.24	6.37
Naive	3	16932	9.04	3.37
TCM	3	39341	21.38	1.57
TEM	3	5081	2.71	3.21
TEMRA	3	200	0.10	17.08
CD8+ T cells	3	18269	9.89	2.73
Naive	3	6769	3.69	1.88
TCM	3	10051	5.37	3.63
TEM	3	845	0.45	10.21
TEMRA	3	697	0.38	3.39
NK cells	3	18407	10.06	1.89
CD16+	3	16616	9.11	3.01
CD16-	3	747	0.37	39.52
CD56+	3	1101	0.58	5.59
Dendritic Cells	3	1925	1.02	16.50

cDC	3	1218	0.69	18.88
pDC	3	603	0.31	15.49
tDC	0*	*	*	*
Monocytes	3	35666	19.09	1.89
cMono	3	17699	9.60	1.27
inMono	3	6625	3.46	3.24
ncMono	3	11363	6.04	5.22
MoMDSCs	3	2200	1.18	3.89
Donor 1 Average				**7.89**

**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 mast cells and tDCs from analysis.*

Table 9: Summary of Inter-Run Precision Assessment for Donor 2 (SBC-2)

Cell Subset	# Replicates	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (%)
Granulocytes				
Eosinophils	3	8310	1.70	5.81
Basophils	3	1846	0.37	2.89
Mast Cells	0*	*	*	*
Neutrophils	3	274701	56.18	0.84
Non-Granulocytes				
B cells	3	10540	5.59	5.15
Naive	3	8651	4.61	6.26
Memory	3	1810	0.96	0.32
PB	0*	*	*	*
T cells	3	107216	56.65	0.60
γδ T cells	3	22140	11.69	1.61
NKT	3	4129	2.18	1.92
DNT	3	4935	2.65	4.29
DPT	3	370	0.20	19.18
CD4+ T cells	3	47272	24.85	1.23
Treg	3	2144	1.10	6.07
Naive	3	21552	11.15	2.43
TCM	3	20858	11.09	2.60
TEM	3	2143	1.15	1.72
TEMRA	0*	*	*	*
CD8+ T cells	3	28370	15.09	1.81
Naive	3	16234	8.48	1.57

TCM	3	9373	4.85	5.62
TEM	3	1580	0.82	9.48
TEMRA	3	1843	0.95	5.42
NK cells	3	22178	11.72	0.50
CD16+	3	12625	6.35	5.78
CD16-	3	9630	4.91	7.12
CD56+	3	698	0.35	16.87
Dendritic Cells	3	2476	1.35	10.93
cDC	3	1769	0.95	12.99
pDC	3	672	0.36	9.85
tDC	1*	*	*	*
Monocytes	3	29450	15.41	1.76
cMono	3	18579	9.67	5.01
inMono	3	6738	3.51	2.94
ncMono	3	4616	2.24	15.38
MoMDSCs	3	2350	1.21	3.63
Donor 2 Average				**5.44**

**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 mast cells, plasmablasts, CD4+ TEMRA and tDCs from analysis.*

Table 10: Summary of Inter-Run Precision Assessment for Donor 3 (SBC-3)

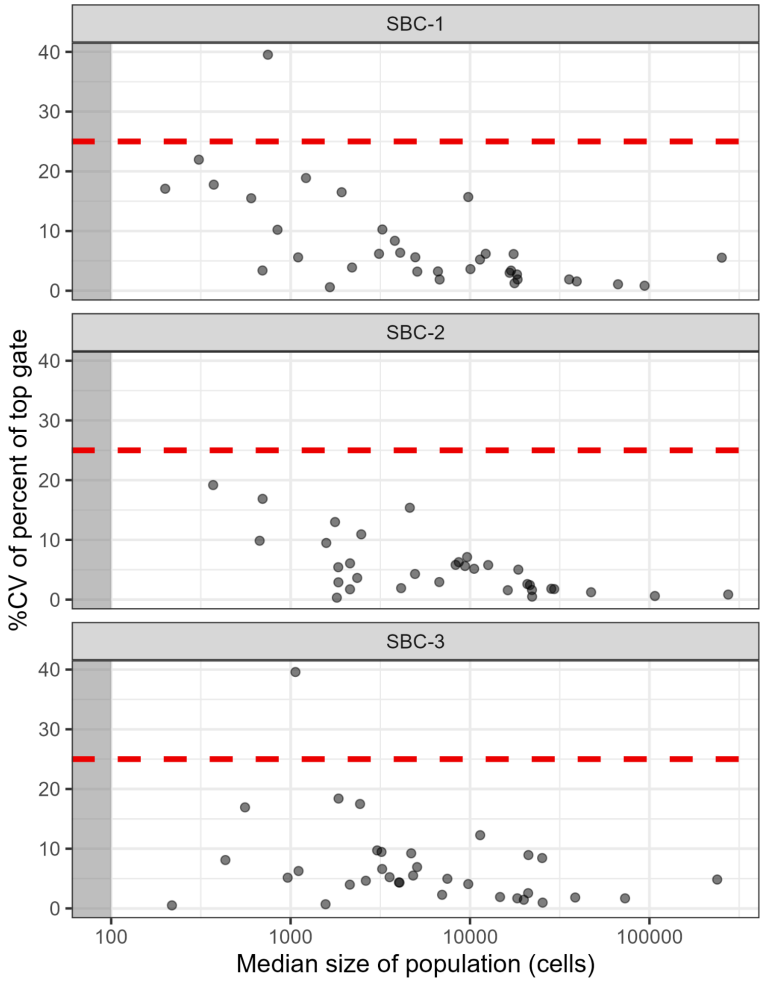
Cell Subset	# Replicates	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (%)
Granulocytes				
Eosinophils	3	25231	5.47	8.43
Basophils	3	4817	1.17	5.50
Mast Cells	0*	*	*	*
Neutrophils	3	238536	56.70	4.84
Non-Granulocytes				
B cells	3	7474	5.58	4.96
Naive	3	4015	2.95	4.36
Memory	3	3237	2.48	6.60
PB	3	218	0.15	0.51
T cells	3	73104	50.80	1.69
γδ T cells	3	4046	2.77	4.33
NKT	3	1107	0.76	6.27
DNT	3	3035	1.96	9.72

DPT	3	963	0.66	5.15
CD4+ T cells	3	38564	26.75	1.83
Treg	3	3561	2.56	5.23
Naive	3	9773	6.73	4.08
TCM	3	21076	14.56	2.55
TEM	3	2625	1.81	4.64
TEMRA	0*	*	*	*
CD8+ T cells	3	25389	17.89	0.97
Naive	3	6987	4.90	2.28
TCM	3	14698	10.39	1.91
TEM	3	2138	1.50	3.98
TEMRA	3	1566	1.10	0.70
NK cells	3	19942	13.91	1.45
CD16+	3	18335	12.88	1.69
CD16-	3	1064	0.74	39.59
CD56+	3	433	0.28	8.10
Dendritic Cells	3	2437	1.88	17.49
cDC	3	1850	1.42	18.40
pDC	3	557	0.43	16.92
tDC	0*	*	*	*
Monocytes	3	21154	16.38	8.94
cMono	3	11388	8.96	12.27
inMono	3	4700	3.62	9.23
ncMono	3	5070	3.81	6.92
MoMDSCs	3	3211	2.55	9.46
Donor 3 Average				**7.09**

**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 mast cells, CD4+ TEMRA and tDCs from analysis.*

Data for individual replicates can be found in DCS131 Appendix A CyTOF TokuKit SH Performance Validation Report Data.

Figure 9: Coefficient of Variation in Frequency (Percentage of Top Gate, which is Leukocytes or Non-Granulocytes) and Median Number of Cells per Immune Population for Inter-Run Precision Assessment



c. *Inter-Instrument Precision*

Inter-instrument precision was assessed by taking whole blood from three (3) healthy donors (SBC-1, SBC-2 and SBC-3) processed with Teiko's TokuKit, and splitting each sample into two replicates. One operator processed all samples at the same time, but ran one set of replicates on Helios #1 and the other set of replicates on Helios #2. Change in immune frequencies between instruments was calculated for precision analysis and can be seen in Table 11 and Figure 10. All measurable (>100 cells median) cell populations passed the acceptable threshold of ≤25% change in frequency between

operators. **Total average inter-instrument % change in frequency for all measurable populations was -1.28%**.

Table 11: Summary of Inter-Instrument Precision Assessment

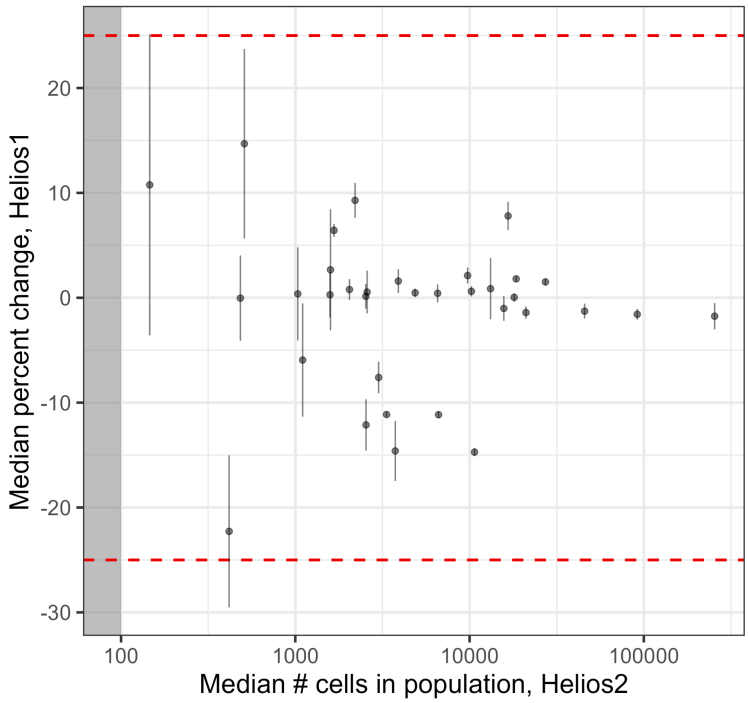
Cell Subset	# Subjects	Median # of cells Helios#2	Median # of cells Helios #1	Average frequency (% top gate) Helios #2	Average frequency (% top gate) Helios #1	Change between instruments (%)
Granulocytes						
Eosinophils	3	10677	9591	2.29	1.95	-14.72
Basophils	3	3339	3125	0.72	0.64	-11.13
Mast Cells	0*	*	*	*	*	*
Neutrophils	3	254865	271740	56.08	55.34	-1.76
Non-Granulocytes						
B cells	3	10243	10431	5.68	5.71	0.61
Naive	3	4870	4953	2.91	2.90	0.47
Memory	3	2583	2748	2.45	2.54	0.55
PB	2	146	166	0.14	0.15	10.76
T cells	3	91675	91339	51.44	50.63	-1.58
γδ T cells	3	3007	2852	2.85	2.64	-7.60
NKT	3	1582	1606	0.89	0.89	0.28
DNT	3	3747	3239	2.13	1.80	-14.61
DPT	3	417	310	0.23	0.17	-22.28
CD4+ T cells	3	45721	45526	26.25	26.38	-1.27
Treg	3	2539	2644	2.26	2.26	0.14
Naive	3	15724	15755	8.82	8.73	-1.02
TCM	3	21063	20858	14.64	14.59	-1.41
TEM	3	2045	2140	1.84	1.85	0.79
TEMRA	1*	*	*	*	*	*
CD8+ T cells	3	18508	19484	15.10	15.37	1.80
Naive	3	6558	6640	4.65	4.77	0.43
TCM	3	9745	10051	5.47	5.57	2.12
TEM	3	1592	1657	0.88	0.91	2.67
TEMRA	3	1033	1195	0.89	0.89	0.37
NK cells	3	18030	18258	11.78	11.67	0.03
CD16+	3	13195	13662	8.93	9.02	0.87
CD16-	3	1102	1064	1.05	0.98	-5.94
CD56+	3	484	544	0.27	0.30	-0.03
Dendritic Cells	3	2203	2437	1.39	1.52	9.29
cDC	3	1667	1850	1.03	1.09	6.43

pDC	3	510	603	0.32	0.37	14.68
tDC	0*	*	*	*	*	*
Monocytes	3	27241	28248	17.63	17.90	1.51
cMono	3	16634	17508	9.43	10.18	7.80
inMono	3	6637	6008	3.84	3.39	-11.15
ncMono	3	3900	4050	3.70	3.74	1.59
MoMDSCs	3	2546	2124	1.41	1.16	-12.13
Average						**-1.28**

**Populations with fewer than 100 cell events were excluded from analysis. Populations in which fewer than 2 replicates had sufficient cell count for analysis were excluded from precision analysis and percentage change calculations. As a result, we excluded mast cells, CD4+ TEMRA and tDCs from analysis.*

Data for individual samples can be found in DCS131 Appendix A CyTOF TokuKit SH Performance Validation Report Data.

Figure 10: Median Percentage of Change in Frequency (Percentage of Leukocytes or Non-Granulocytes) and Median Number of Cells per Immune Population for Inter-Instrument Assessment



d. *Inter-Kit Precision*

Inter-kit precision was assessed by collecting whole blood from three (3) healthy donors (SBC-1, SBC-2 and SBC-3) and splitting each blood sample into three replicates. Three different operators (J.J., T.L. and H.W.) each received one replicate from each donor and processed all replicates with an individual TokuKit SH (resulting in three TokuKits per donor and nine TokuKits total). All replicates were subsequently processed and run on CyTOF by a different, fourth operator. Variance between individual donors, as processed by different operators with different TokuKits, was calculated for precision analysis and can be seen in Tables 12-14 and Figure 11. All measurable (>100 cells median) cell populations passed the acceptable threshold of ≤25% variation between kits. **Total average inter-kit %CV for all measurable populations across all three donors was 5.76%**, with individual donor average %CVs of 6.00%, 3.42%, and 7.79% for Donor 1 (SBC-1), Donor 2 (SBC-2), and Donor 3 (SBC-3), respectively. In Donor 1 (SBC-1), eosinophil has 33.00% CV, but the average %CV for eosinophils across all three donors is 15.94.

Table 12: Summary of Inter-Kit Precision Assessment for Donor 1 (SBC-1)

Cell Subset	# Replicates (Kits)	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (kits) (%)
Granulocytes				
Eosinophils	3	9773	2.43	33.00
Basophils	3	3190	0.68	2.35
Mast Cells	0*	*	*	*
Neutrophils	3	253012	53.38	6.17
Non-Granulocytes				
B cells	3	16394	8.78	5.61
Naive	3	4654	2.45	5.58
Memory	3	11420	6.16	6.28
PB	3	259	0.16	14.12
T cells	3	93904	50.47	0.69
γδ T cells	3	2979	1.63	2.06
NKT	3	1655	0.90	5.98
DNT	3	3968	2.13	0.54
DPT	3	451	0.23	16.41
CD4+ T cells	3	66782	35.87	0.54
Treg	3	3861	2.10	0.86
Naive	3	16932	9.11	1.20
TCM	3	39341	21.22	0.72
TEM	3	4955	2.70	2.18

TEMRA	3	175	0.10	10.99
CD8+ T cells	3	17938	9.72	1.15
Naive	3	6769	3.64	2.15
TCM	3	9687	5.26	1.68
TEM	3	785	0.42	1.55
TEMRA	3	697	0.39	3.82
NK cells	3	18407	10.08	2.25
CD16+	3	16616	9.12	2.54
CD16-	3	658	0.36	10.08
CD56+	3	1020	0.58	5.59
Dendritic Cells	3	1497	0.89	9.86
cDC	3	999	0.59	8.00
pDC	3	468	0.28	15.69
tDC	0*	*	*	*
Monocytes	3	32480	18.64	5.12
cMono	3	15994	9.22	5.44
inMono	3	6053	3.46	4.90
ncMono	3	10439	5.97	4.85
MoMDSCs	3	1830	1.12	9.90
Donor 1 Average				**6.00**

**Populations with fewer than 100 cell events were excluded from analysis. As a result, we excluded mast cells and tDCs from analysis.*

Table 13: Summary of Inter-Kit Precision Assessment for Donor 2 (SBC-2)

Cell Subset	# Replicates (Kits)	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (kits) (%)
Granulocytes				
Eosinophils	3	8310	1.87	11.76
Basophils	3	1582	0.37	5.98
Mast Cells	0*	*	*	*
Neutrophils	3	238222	56.98	2.93
Non-Granulocytes				
B cells	3	8006	4.88	7.77
Naive	3	6433	3.96	7.64
Memory	3	1553	0.91	9.71
PB	0*	*	*	*
T cells	3	91441	55.80	0.79
γδ T cells	3	19655	11.84	0.94
NKT	3	3544	2.17	2.62

DNT	3	4818	2.89	3.89
DPT	3	383	0.23	10.71
CD4+ T cells	3	39188	23.97	2.08
Treg	3	1612	1.00	5.60
Naive	3	17696	10.80	2.43
TCM	3	17426	10.67	1.62
TEM	3	1847	1.13	0.55
TEMRA	0*	*	*	*
CD8+ T cells	3	23895	14.69	1.01
Naive	3	13566	8.28	1.21
TCM	3	7402	4.62	2.21
TEM	3	1257	0.80	4.41
TEMRA	3	1670	1.00	2.15
NK cells	3	19270	11.69	0.33
CD16+	3	10704	6.40	1.47
CD16-	3	7893	4.86	1.08
CD56+	3	508	0.33	6.06
Dendritic Cells	3	2070	1.24	1.01
cDC	3	1462	0.88	0.83
pDC	3	553	0.33	3.15
tDC	0*	*	*	*
Monocytes	3	24684	14.96	1.24
cMono	3	15064	9.13	1.63
inMono	3	5949	3.57	1.27
ncMono	3	3681	2.26	1.50
MoMDSCs	3	2203	1.30	5.45
Donor 2 Average				**3.42**

**Populations with fewer than 100 cell events were excluded from analysis. As a result, we excluded mast cells, plasmablasts, CD4+ TEMRA and tDCs from analysis.*

Table 14: Summary of Inter-Kit Precision Assessment for Donor 3 (SBC-3)

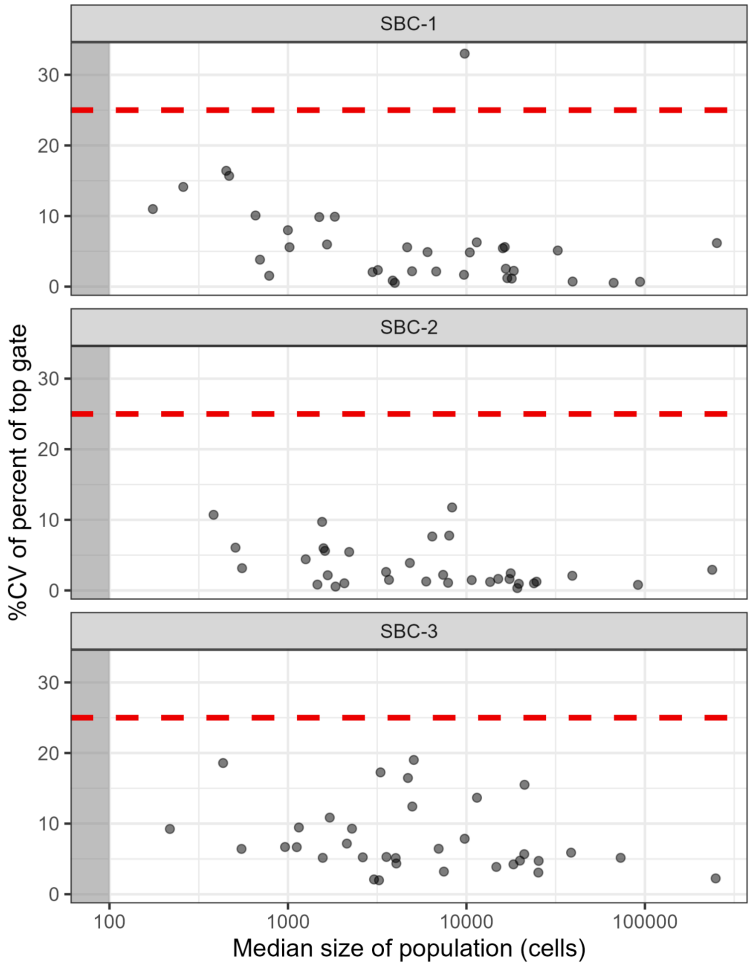
Cell Subset	# Replicates (Kits)	Median # of cells measured	Average % of leukocytes / non-granulocytes	Coefficient of Variation between replicates (kits) (%)
Granulocytes				
Eosinophils	3	25285	5.86	3.07
Basophils	3	4973	1.24	12.42
Mast Cells	0*	*	*	*
Neutrophils	3	249071	58.03	2.24
Non-Granulocytes				

B cells	3	7474	5.25	3.21
Naive	3	4015	2.78	5.12
Memory	3	3237	2.33	1.98
PB	3	218	0.14	9.24
T cells	3	73104	49.32	5.16
γδ T cells	3	4046	2.75	4.35
NKT	3	1120	0.79	6.67
DNT	3	3035	2.11	2.09
DPT	3	963	0.68	6.69
CD4+ T cells	3	38564	25.82	5.89
Treg	3	3561	2.40	5.28
Naive	3	9773	6.51	7.86
TCM	3	21076	14.10	5.67
TEM	3	2625	1.75	5.23
TEMRA	0*	*	*	*
CD8+ T cells	3	25389	17.17	4.72
Naive	3	6987	4.72	6.44
TCM	3	14698	9.97	3.86
TEM	3	2138	1.42	7.17
TEMRA	3	1566	1.06	5.15
NK cells	3	19942	13.58	4.75
CD16+	3	18335	12.52	4.23
CD16-	3	1151	0.77	9.45
CD56+	3	433	0.29	18.58
Dendritic Cells	3	2284	1.75	9.28
cDC	3	1714	1.32	10.85
pDC	3	549	0.40	6.42
tDC	0*	*	*	*
Monocytes	3	21154	16.83	15.50
cMono	3	11453	9.06	13.67
inMono	3	4700	3.69	16.46
ncMono	3	5070	4.10	19.02
MoMDSCs	3	3300	2.64	17.26
Donor 3 Average				**7.79**

**Populations with fewer than 100 cell events were excluded from analysis. As a result, we excluded mast cells, CD4+ TEMRA and tDCs from analysis.*

Data for individual replicates can be found in DCS131 Appendix A CyTOF TokuKit SH Performance Validation Report Data.

Figure 11: Coefficient of Variation in Frequency (Percentage of Leukocytes or Non-Granulocytes) and Median Number of Cells per Immune Population for Inter-Kit Precision Assessment



3. Stability
   a. *Pre-Fixation Stability*
      Pre-fixation stability was assessed by taking whole blood collected in sodium heparin vacutainers from 3 (three) healthy donors (D1H, D2H and D3H). The vacutainers containing blood were stored at room temperature. 2 mL of blood from each donor was processed using the TokuKit within three hours of collection (t=0), and then at regular intervals after the initial processing time point : t=2 hours, t=4 hours, t=6 hours, t=24 hours and t=48 hours, for a total of 6 timepoints. All samples were processed, stained

and run by the same operator. Change in immune frequencies compared to t=0 was calculated for stability analysis and can be seen in Tables 15-19 and Figures 12-16. **Only the t=2 hours timepoint met the acceptance criteria of ≤25% change for ≥95% of all measurable (>100 cell events) immune populations from t = 0 hours, with an average % of change from t=0 hours of 0.12%.** The percentage of measurable immune populations with ≤25% change was 100%, 88%, 85%, 82% and 50% for 2 hours, 4 hours, 6 hours, 24 hours and 48 hours, respectively. The measurable populations with >25% change were total monocytes, cMono, inMono and MoMDSCs from 4 hours onwards; CD16- NK cells from 6 hours onwards; DPT from 24 hours onwards; and neutrophils, total B cells, naive B cells, NKT cells, DNT cells, CD4+ TEMRA cells, CD8+ TEMRA cells, CD56+ NK cells, total DCs, cDCs and ncMono at 48 hours.

In light of these findings, all blood samples isolated with sodium heparin vacutainers will be processed within 5 hours of collection to preserve sample integrity.

Table 15: Summary of Pre-Fixation Stability Assessment for 2 hours Post-Blood Collection

Cell Subset	# Subjects	Median # of cells 0h	Median # of cells 2h	Median % of Leukocytes/ Non-Granulocytes 0h	Median % of Leukocytes/ Non-Granulocytes 2h	Change between time points (%)
Granulocytes						
Eosinophils	3	16850	20548	5.10	4.73	-7.11
Basophils	3	5222	3581	1.34	1.23	-4.90
Mast Cells	0*	*	*	*	*	*
Neutrophils	3	144125	190774	43.60	43.96	5.00
Non-Granulocytes						
B cells	3	18170	12629	7.88	7.79	-1.11
Naive	3	14055	10113	6.28	6.24	-0.61
Memory	3	3679	2459	1.81	1.72	-3.35
PB	0*	*	*	*	*	*
T cells	3	99135	105407	62.26	64.94	2.20
γδ T cells	3	1513	2071	0.98	0.99	3.24
NKT	3	1469	1848	1.55	1.70	16.39
DNT	3	2130	2892	1.34	1.39	-6.41
DPT	3	349	347	0.15	0.21	0.76
CD4+ T cells	3	62583	65968	39.30	40.05	1.90
Treg	3	2405	3369	2.08	2.19	4.99
Naive	3	15661	20645	12.72	12.41	0.70
TCM	3	30912	35243	20.24	21.06	4.06

TEM	3	2218	1933	1.78	1.78	0.12
TEMRA	2	130	172	0.14	0.16	-0.01
CD8+ T cells	3	19213	26098	12.07	12.52	2.27
Naive	3	4545	5936	3.22	3.29	0.36
TCM	3	11082	15171	6.96	7.54	3.80
TEM	3	1262	949	0.54	0.59	-2.09
TEMRA	3	373	428	0.37	0.39	6.84
NK cells	3	14551	16440	10.34	10.50	-1.14
CD16+	3	13595	15327	9.56	9.53	-1.04
CD16-	3	296	274	0.31	0.25	-19.36
CD56+	3	649	829	0.45	0.48	11.28
Dendritic Cells	3	843	1360	0.72	0.84	18.98
cDC	3	611	981	0.51	0.59	21.42
pDC	3	218	330	0.15	0.20	23.69
tDC	0*	*	*	*	*	*
Monocytes	3	25036	19825	15.72	12.73	-15.32
cMono	3	13605	11132	8.54	6.73	-15.77
inMono	3	7728	6190	4.85	3.85	-18.41
ncMono	3	3711	4515	2.33	2.17	-7.06
MoMDSCs	3	506	464	0.53	0.43	-20.11
Total average 2h						**0.12**

**Populations with fewer than 100 cell events at time point 0 hours were excluded from analysis. Populations in which fewer than 2 subjects had sufficient cell count for analysis were excluded from stability analysis and change calculations. As a result, we excluded mast cells, plasmablasts and tDCs from analysis.*

Figure 12: Median Percentage of Change in Frequency (Percentage of Leukocytes or Non-Granulocytes) and Median Number of Cells per Immune Population for Pre-Fixation Stability Assessment at 2 hours compared to 0 hours

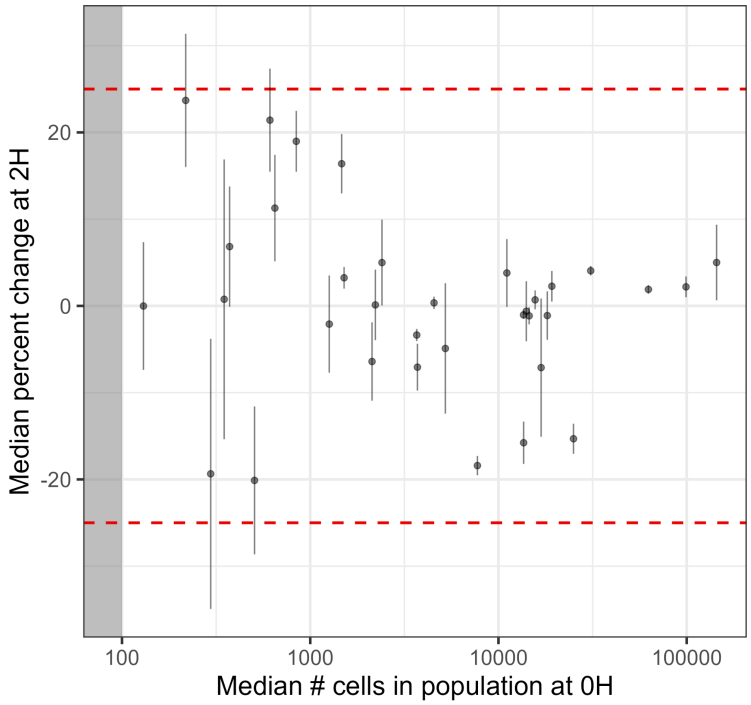


Table 16: Summary of Pre-Fixation Stability Assessment for 4 hours Post-Blood Collection

Cell Subset	# Subjects	Median # of cells 0h	Median # of cells 4h	Median % of Leukocytes/ Non-Granulocytes 0h	Median % of Leukocytes/ Non-Granulocytes 4h	Change between time points (%)
Granulocytes						
Eosinophils	3	16850	23096	5.10	4.99	-2.16
Basophils	3	5222	4095	1.34	1.41	-1.16
Mast Cells	0*	*	*	*	*	*
Neutrophils	3	144125	195173	43.60	42.14	-1.71
Non-Granulocytes						
B cells	3	18170	15016	7.88	8.66	9.06
Naive	3	14055	12045	6.28	6.95	10.02
Memory	3	3679	2901	1.81	1.92	6.31
PB	0*	*	*	*	*	*
T cells	3	99135	115164	62.26	66.45	7.43

γδ T cells	3	1513	2300	0.98	1.03	5.36
NKT	3	1469	1660	1.55	1.73	11.16
DNT	3	2130	3292	1.34	1.45	8.55
DPT	3	349	316	0.15	0.18	7.31
CD4+ T cells	3	62583	71898	39.30	41.48	5.83
Treg	3	2405	3582	2.08	2.07	0.54
Naive	3	15661	22749	12.72	13.63	3.72
TCM	3	30912	38126	20.24	22.00	8.83
TEM	3	2218	1935	1.78	2.01	10.08
TEMRA	2	130	157	0.14	0.16	15.06
CD8+ T cells	3	19213	29670	12.07	13.09	6.47
Naive	3	4545	7147	3.22	3.68	10.44
TCM	3	11082	16368	6.96	7.67	4.71
TEM	3	1262	1003	0.54	0.58	1.94
TEMRA	3	373	465	0.37	0.48	13.09
NK cells	3	14551	15688	10.34	10.73	5.85
CD16+	3	13595	14631	9.56	9.79	5.86
CD16-	3	296	327	0.31	0.24	-13.66
CD56+	3	649	790	0.45	0.48	13.24
Dendritic Cells	3	843	974	0.72	0.75	-1.22
cDC	3	611	646	0.51	0.52	-9.30
pDC	3	218	304	0.15	0.18	0.52
tDC	0*	*	*	*	*	*
Monocytes	3	25036	17105	15.72	9.87	-35.57
cMono	3	13605	7459	8.54	4.65	-38.09
inMono	3	7728	4192	4.85	2.57	-42.29
ncMono	3	3711	4461	2.33	1.97	-12.16
MoMDSCs	3	506	267	0.53	0.28	-48.48
Total average 4h						**-1.01**

**Populations with fewer than 100 cell events at time point 0 hours were excluded from analysis. Populations in which fewer than 2 subjects had sufficient cell count for analysis were excluded from stability analysis and change calculations. As a result, we excluded mast cells, plasmablasts and tDCs from analysis.*

Figure 13: Median Percentage of Change in Frequency (Percentage of Leukocytes or Non-Granulocytes) and Median Number of Cells per Immune Population for Pre-Fixation Stability Assessment at 4 hours compared to 0 hours

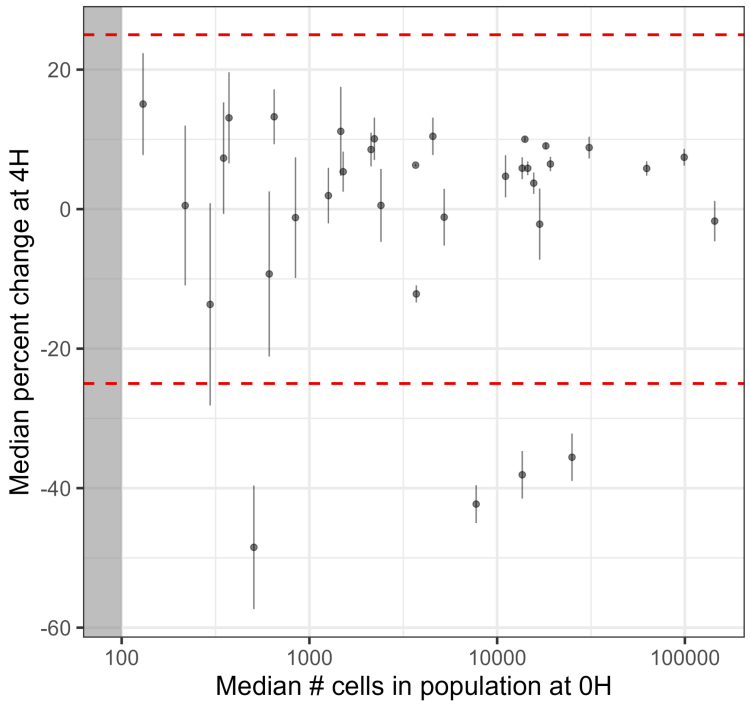


Table 17: Summary of Pre-Fixation Stability Assessment for 6 hours Post-Blood Collection

Cell Subset	# Subjects	Median # of cells 0h	Median # of cells 6h	Median % of Leukocytes/ Non-Granulocytes 0h	Median % of Leukocytes/ Non-Granulocytes 6h	Change between time points (%)
Granulocytes						
Eosinophils	3	16850	18550	5.10	4.56	-10.62
Basophils	3	5222	5140	1.34	1.26	-3.51
Mast Cells	0*	*	*	8	8	8
Neutrophils	3	144125	168236	43.60	41.32	5.85
Non-Granulocytes						
B cells	3	18170	18700	7.88	8.63	9.47
Naive	3	14055	15044	6.28	6.94	10.56
Memory	3	3679	3592	1.81	1.80	0.75
PB	0*	*	*	*	*	*
T cells	3	99135	140960	62.26	66.72	4.99

γδ T cells	3	1513	2115	0.98	1.03	1.18
NKT	3	1469	1960	1.55	1.85	19.39
DNT	3	2130	2660	1.34	1.30	-2.76
DPT	3	349	550	0.15	0.25	18.64
CD4+ T cells	3	62583	87370	39.30	41.55	5.30
Treg	3	2405	3533	2.08	2.08	0.10
Naive	3	15661	21075	12.72	13.24	4.79
TCM	3	30912	45213	20.24	21.79	7.69
TEM	3	2218	2158	1.78	1.92	5.20
TEMRA	2	130	171	0.14	0.16	1.23
CD8+ T cells	3	19213	26936	12.07	13.17	5.15
Naive	3	4545	6648	3.22	3.49	8.18
TCM	3	11082	15762	6.96	7.71	3.01
TEM	3	1262	1128	0.54	0.52	-3.35
TEMRA	3	373	407	0.37	0.38	4.35
NK cells	3	14551	15995	10.34	10.88	-0.41
CD16+	3	13595	14945	9.56	9.92	0.27
CD16-	3	296	249	0.31	0.22	-27.11
CD56+	3	649	813	0.45	0.45	12.10
Dendritic Cells	3	843	832	0.72	0.72	0.26
cDC	3	611	566	0.51	0.50	-6.03
pDC	3	218	323	0.15	0.17	15.37
tDC	0*	*	*	*	*	*
Monocytes	3	25036	16406	15.72	9.40	-36.02
cMono	3	13605	7999	8.54	3.90	-40.26
inMono	3	7728	4608	4.85	2.26	-45.13
ncMono	3	3711	3831	2.33	1.87	-16.17
MoMDSCs	3	506	442	0.53	0.42	-27.12
Total average 6h						**-2.20**

**Populations with fewer than 100 cell events at time point 0 hours were excluded from analysis. Populations in which fewer than 2 subjects had sufficient cell count for analysis were excluded from stability analysis and change calculations. As a result, we excluded mast cells, plasmablasts and tDCs from analysis.*

Figure 14: Median Percentage of Change in Frequency (Percentage of Live Cells or Non-Granulocytes) and Median Number of Cells per Immune Population for Pre-Fixation Stability Assessment at 6 hours compared to 0 hours

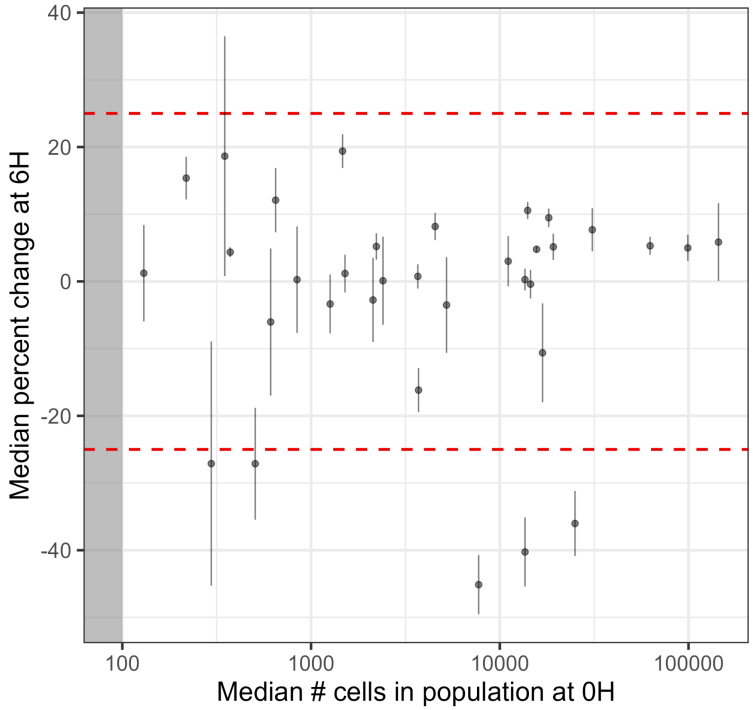


Table 18: Summary of Pre-Fixation Stability Assessment for 24 hours Post-Blood Collection

Cell Subset	# Subjects	Median # of cells 0h	Median # of cells 24h	Median % of Leukocytes/ Non-Granulocytes 0h	Median % of Leukocytes/ Non-Granulocytes 24h	Change between time points (%)
Granulocytes						
Eosinophils	3	16850	15919	5.10	4.64	-14.90
Basophils	3	5222	4694	1.34	1.35	1.12
Mast Cells	0*	*	*	*	*	*
Neutrophils	3	144125	120513	43.60	35.09	-5.30
Non-Granulocytes						
B cells	3	18170	18270	7.88	8.74	20.64
Naive	3	14055	14749	6.28	7.06	23.93
Memory	3	3679	3488	1.81	2.07	14.45
PB	0*	*	*	*	*	*
T cells	3	99135	127194	62.26	67.73	6.41

γδ T cells	3	1513	1980	0.98	1.06	6.39
NKT	3	1469	1890	1.55	2.06	24.75
DNT	3	2130	2945	1.34	1.58	16.71
DPT	3	349	555	0.15	0.27	28.81
CD4+ T cells	3	62583	77786	39.30	41.72	5.58
Treg	3	2405	3431	2.08	2.10	7.47
Naive	3	15661	19016	12.72	12.65	1.44
TCM	3	30912	38779	20.24	20.80	6.18
TEM	3	2218	2513	1.78	2.00	12.31
TEMRA	2	130	153	0.14	0.17	10.91
CD8+ T cells	3	19213	24468	12.07	13.12	8.11
Naive	3	4545	6715	3.22	3.76	16.51
TCM	3	11082	13631	6.96	7.31	2.89
TEM	3	1262	1276	0.54	0.61	13.35
TEMRA	3	373	403	0.37	0.44	16.48
NK cells	3	14551	16669	10.34	11.02	17.31
CD16+	3	13595	15310	9.56	9.76	14.75
CD16-	3	296	578	0.31	0.55	79.58
CD56+	3	649	842	0.45	0.56	24.64
Dendritic Cells	3	843	1021	0.72	0.69	3.44
cDC	3	611	773	0.51	0.47	8.05
pDC	3	218	199	0.15	0.14	-22.04
tDC	0*	*	*	*	*	*
Monocytes	3	25036	10639	15.72	8.19	-49.69
cMono	3	13605	5396	8.54	3.96	-51.70
inMono	3	7728	2948	4.85	1.79	-54.03
ncMono	3	3711	3416	2.33	1.83	-23.22
MoMDSCs	3	506	111	0.53	0.12	-84.25
Total average 24h						**2.56**

**Populations with fewer than 100 cell events at time point 0 hours were excluded from analysis. Populations in which fewer than 2 subjects had sufficient cell count for analysis were excluded from stability analysis and change calculations. As a result, we excluded mast cells, plasmablasts and tDCs from analysis.*

Figure 15: Median Percentage of Change in Frequency (Percentage of Leukocytes or Non-Granulocytes) and Median Number of Cells per Immune Population for Pre-Fixation Stability Assessment at 24 hours compared to 0 hours

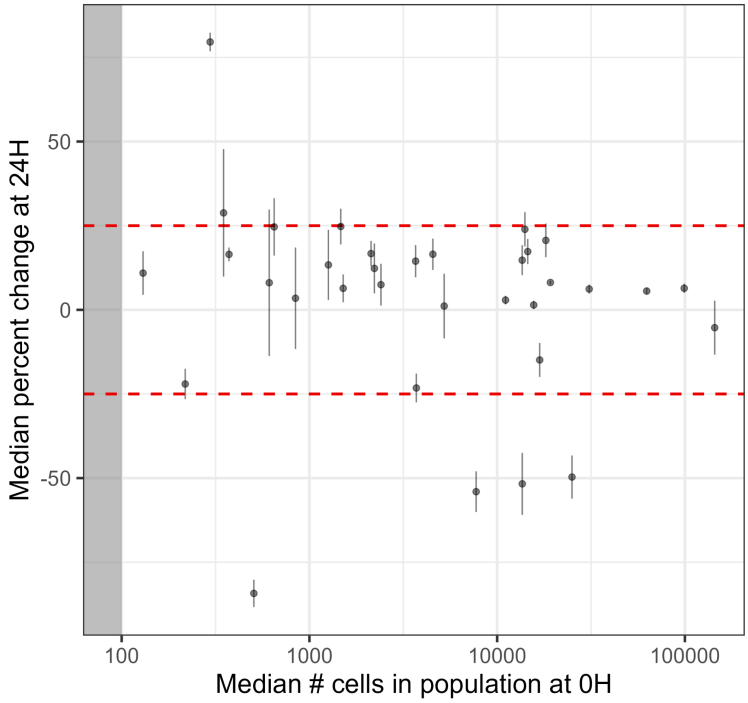


Table 19: Summary of Pre-Fixation Stability Assessment for 48 hours Post-Blood Collection

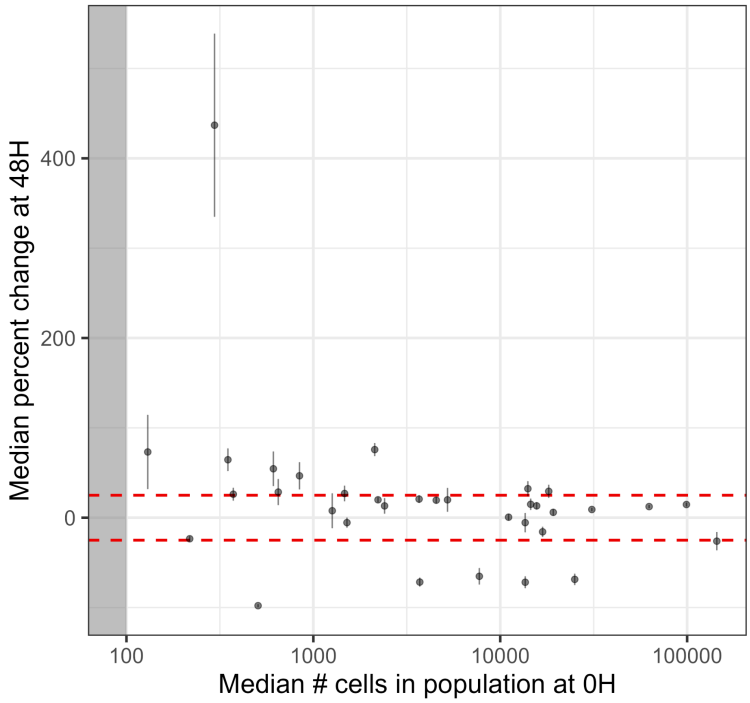
Cell Subset	# Subjects	Median # of cells 0h	Median # of cells 48h	Median % of Leukocytes/ Non-Granulocytes 0h	Median % of Leukocytes/ Non-Granulocytes 48h	Change between time points (%)
Granulocytes						
Eosinophils	3	16850	7937	5.10	4.86	-15.71
Basophils	3	5222	2728	1.34	1.53	19.88
Mast Cells	0*	*	*	*	*	*
Neutrophils	3	144125	60465	43.60	26.63	-26.10
Non-Granulocytes						
B cells	3	18170	14307	7.88	8.55	29.40
Naive	3	14055	11384	6.28	6.80	32.28
Memory	3	3679	2902	1.81	2.25	20.74
PB	0*	*	*	*	*	*
T cells	3	99135	74059	62.26	69.97	14.66

γδ T cells	3	1513	1032	0.98	0.99	-5.47
NKT	3	1469	1097	1.55	2.07	26.95
DNT	3	2130	2542	1.34	2.45	75.79
DPT	3	349	410	0.15	0.25	64.51
CD4+ T cells	3	62583	45817	39.30	43.76	12.36
Treg	3	2405	2092	2.08	2.18	13.14
Naive	3	15661	11318	12.72	15.75	13.21
TCM	3	30912	22192	20.24	22.00	9.14
TEM	3	2218	1993	1.78	2.13	19.93
TEMRA	2	130	163	0.14	0.24	73.17
CD8+ T cells	3	19213	13266	12.07	12.79	5.98
Naive	3	4545	3686	3.22	3.85	19.48
TCM	3	11082	7262	6.96	7.00	0.58
TEM	3	1262	971	0.54	0.63	7.75
TEMRA	3	373	336	0.37	0.51	26.15
NK cells	3	14551	9360	10.34	9.97	14.93
CD16+	3	13595	8045	9.56	6.63	-5.50
CD16-	3	296	1424	0.31	1.56	436.86
CD56+	3	649	476	0.45	0.58	28.55
Dendritic Cells	3	843	590	0.72	1.11	46.64
cDC	3	611	466	0.51	0.88	54.47
pDC	3	218	112	0.15	0.11	-23.43
tDC	0*	*	*	*	*	*
Monocytes	3	25036	3480	15.72	5.28	-68.62
cMono	3	13605	1815	8.54	2.24	-71.84
inMono	3	7728	1287	4.85	1.99	-65.21
ncMono	3	3711	576	2.33	0.75	-71.64
MoMDSCs	3	506	19	0.53	0.02	-97.90
Total average 48h						**18.09**

**Populations with fewer than 100 cell events at time point 0 hours were excluded from analysis. Populations in which fewer than 2 subjects had sufficient cell count for analysis were excluded from stability analysis and change calculations. As a result, we excluded mast cells, plasmablasts and tDCs from analysis.*

Data for individual replicates can be found in DCS131 Appendix A CyTOF TokuKit SH Performance Validation Report Data.

Figure 16: Median Percentage of Change in Frequency (Percentage of Leukocytes or Non-Granulocytes) and Median Number of Cells per Immune Population for Pre-Fixation Stability Assessment at 48 hours compared to 0 hours



b. *Post-Collection Stability*

Post-collection stability was assessed by taking whole blood from 3 (three) healthy donors (D1H, D2H and D3H) and processing them with Teiko's TokuKit. All samples were split into 6 technical replicates and stored at -80C on 11-07-2023. The first set of replicates was thawed the next day, 11-08-2023, and run on CyTOF that same day, t=0. Another set of replicates was thawed and run on CyTOF on 12-08-2023, t=1 month. All steps were performed by the same operator. Change in immune frequencies compared to t=0 was calculated for stability analysis and can be seen in Table 20 and Figure 17. At 1 month, **97% of all measurable (>100 cell events) immune populations met the acceptance criteria of ≤25% change from t = 0 hours.** The average % change across measurable populations from t=0 was 3.61%. At t=1 month, moMDSCs had a %CV of 44.35%.

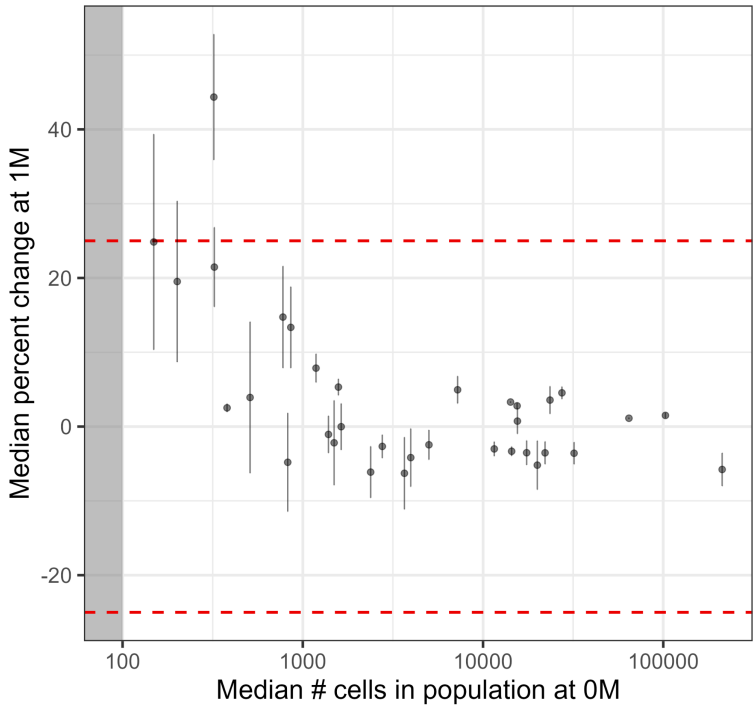
Table 20: Summary of Post-Collection Stability Assessment for 1 Month Post-TokuKit Processing

Cell Subset	# Subjects	Median # of cells 0 months	Median # of cells 1 month	Median % of Leukocytes/ Non-Granulocytes 0 months	Median % of Leukocytes/ Non-Granulocytes 1 month	Change between time points (%)
Granulocytes						
Eosinophils	3	20004	14838	4.43	3.81	-5.19
Basophils	3	3663	3310	1.16	1.07	-6.29
Mast Cells	0*	*	*	*	*	*
Neutrophils	3	212144	166313	44.88	42.76	-5.78
Non-Granulocytes						
B cells	3	14410	14912	8.90	8.56	-3.33
Naive	3	11539	11797	7.13	6.77	-3.01
Memory	3	2761	2991	2.28	2.22	-2.67
PB	0*	*	*	*	*	*
T cells	3	102795	112220	61.16	61.58	1.50
γδ T cells	3	2380	1931	1.07	1.10	-6.13
NKT	3	1492	1726	1.57	1.54	-2.19
DNT	3	1184	1100	0.51	0.55	7.87
DPT	3	380	419	0.23	0.24	2.52
CD4+ T cells	3	64483	70272	39.82	40.35	1.12
Treg	3	3972	3448	2.61	2.28	-4.18
Naive	3	23527	20818	14.04	15.22	3.57
TCM	3	31997	34615	19.76	19.88	-3.59
TEM	3	1576	1921	1.66	1.71	5.31
TEMRA	2	149	220	0.16	0.20	24.84
CD8+ T cells	3	27398	24802	11.89	12.45	4.54
Naive	3	7227	6718	3.49	3.53	4.95
TCM	3	15515	13687	6.82	6.87	0.73
TEM	3	858	1046	0.53	0.60	13.35
TEMRA	3	323	464	0.34	0.41	21.46
NK cells	3	15456	18770	11.17	11.48	2.79
CD16+	3	14213	17391	10.07	10.41	3.30
CD16-	3	510	368	0.22	0.20	3.92
CD56+	3	776	1053	0.60	0.61	14.74
Dendritic Cells	3	1633	1401	0.97	1.01	-0.02
cDC	3	1388	1187	0.82	0.84	-1.06
pDC	3	201	205	0.12	0.16	19.52

tDC	0*	*	*	*	*	*
Monocytes	3	22084	22912	16.47	16.30	-3.54
cMono	3	17457	19315	13.76	13.65	-3.52
inMono	3	825	796	0.51	0.49	-4.81
ncMono	3	5010	4224	2.17	2.12	-2.45
MoMDSCs	3	321	548	0.28	0.43	44.35
Total average 1 month						**3.61**

**Populations with fewer than 100 cell events at time point 0 months were excluded from analysis. Populations in which fewer than 2 subjects had sufficient cell count for analysis were excluded from stability analysis and change calculations. As a result, we excluded mast cells, plasmablasts, CD4 TEMRA and tDCs from analysis.*

Figure 17: Median Percentage of Change in Frequency (Percentage of Live Cells or Non-Granulocytes) and Median Number of Cells per Immune Population for Pre-Fixation Stability Assessment at 1 month compared to 0 months.



4. Reference Range

Reference intervals were established by taking whole blood from 6 (six) healthy donors (SBC-1, SBC-2, SBC-3, D1H, D2H and D3H) and processing them with Teiko's TokuKit SH before processing on CyTOF. 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 21.

Reference ranges were established in healthy subjects for all but four populations: mast cells, CD4 TEMRA, plasmablasts and transitional DCs. These are rare cell populations that in healthy individuals are present at low frequencies. Mast cells are typically tissue-resident cells and are only found in the blood in a progenitor state (Fong and Crane, 2023). 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 - meaning the markers that define them are dimly expressed, or there is no clear separation between positive and negative populations. Seong et al. (2017) found that plasmablasts in PBMCs from n=14 healthy donors did not exceed 0.25% of total lymphocytes, and are only elevated in particular clinical settings, such as autoimmune disease 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). 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 21: Reference Range Values for Whole Blood from Healthy Subjects Processed with Teiko's TokuKit SH.

		% of Leukocytes / Non-Granulocytes		
Cell Subset	**# Subjects**	**Median**	**Standard Deviation**	**Range**
Granulocytes				
Eosinophils	6	3.68	2.40	1.79 - 7.4
Basophils	6	0.97	0.46	0.38 - 1.61
Mast Cells	0*	*	*	*
Neutrophils	6	55.01	9.71	34.86 - 59.64
Non-Granulocytes				
B cells	6	6.89	2.39	5.85 - 11.41
Naive	6	4.43	2.28	2.74 - 8.83

Memory	6	2.19	2.13	1 - 6.9
PB	2*	*	*	*
T cells	6	53.84	6.38	48.98 - 63.65
γδ T cells	6	1.58	4.30	0.95 - 11.98
NKT	6	1.21	3.06	0.44 - 8.49
DNT	6	1.96	0.59	0.91 - 2.59
DPT	6	0.20	0.29	0.11 - 0.76
CD4+ T cells	6	36.91	6.82	24.93 - 39.83
Treg	6	2.26	0.59	1.13 - 2.54
Naive	6	10.60	3.03	6.47 - 15.24
TCM	6	19.83	4.31	10.91 - 21.11
TEM	6	1.76	1.94	0.95 - 6.17
TEMRA	3*	*	*	*
CD8+ T cells	6	13.54	4.28	8.73 - 19.66
Naive	6	4.29	3.01	2.85 - 9.94
TCM	6	6.19	2.28	4.67 - 10
TEM	6	0.65	0.45	0.41 - 1.42
TEMRA	6	0.66	0.38	0.16 - 1.04
NK cells	6	11.03	3.10	6.46 - 15.38
CD16+	6	3.71	5.36	0.18 - 12.81
CD16-	6	0.39	1.70	0.17 - 4.48
CD56+	6	3.21	5.87	0.27 - 14.37
Dendritic Cells	6	0.86	0.50	0.53 - 1.92
cDC	6	0.60	0.37	0.38 - 1.4
pDC	6	0.26	0.13	0.14 - 0.47
tDC	0*	*	*	*
Monocytes	6	16.59	2.54	14.08 - 20.94
cMono	6	9.46	1.82	6.52 - 12.17
inMono	6	3.89	1.34	3.47 - 6.99
ncMono	6	3.09	1.57	1.86 - 6.1
MoMDSCs	6	1.03	0.92	0.25 - 2.82

**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 mast cells, CD4+ TEMRA cells, Plasmablasts and Transitional Dendritic Cells from reference range calculations.*

Data for individual donors can be found in DCS131 Appendix A CyTOF TokuKit SH Performance 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 nearly all major immune population subsets within granulocytes (including eosinophils, neutrophils and basophils) and non-granulocytes (including T cells, B cells, NK cells, monocytes and dendritic cells) in whole blood, and was used to set a reference range for nearly all populations in whole blood from healthy subjects. The acceptance criteria for precision within runs (intra-run), between runs (inter-run), between instruments (inter-instrument) and between kits / operators (inter-kit) were met for >95% of all reportable cell populations. In addition, the acceptance criteria for pre-fixation stability up to 2 hours were met for 100% of all reportable cell populations. Finally, post-fixation stability up to 1 month was established for 97% of all reportable cell populations.

9. References (access link)

Appendices

- Appendix A CyTOF TokuKit SH Performance Validation Report Data
- Appendix B CyTOF TokuKit SH Performance Validation Report Data (Functional marker subsets)

Standard Operating Procedures

- DCS006 Preparation of CyTOF reagents
- DCS007B CyTOF Staining with Barcode (eBio perm version)
- DCS034 Quality Assurance and Performance Verification
- DCS130 CyTOF TokuKit SH Performance Validation Plan

Manuals

- TokuKit manual

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
- Fong M, Crane JS. Histology, Mast Cells. [Updated 2023 May 1]. In: *StatPearls [Internet].* Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499904/
- Seong et al. Trafficking receptor signatures define blood plasmablasts responding to tissue-specific immune challenge. *JCI Insight* (2017). 2(6):e90233.

10. History block

Revision	Originator	Date Effective	Nature of Change
01	Jolien Sweere	2023/12/12	Initial release
02	Jolien Sweere	2024/04/02	Removed eosinophils as reported population
03	Jolien Sweere	2024/04/09	Added eosinophils back in as reportable population, but different gating strategy than v01

Reviewed by

Name	Date	Signature
Jacek Klepacki	01/22/2024	
Jacek Klepacki	04/03/2024	
Li-Chun Cheng	2024.4.9	
Jacek Klepacki	04/10/2024