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Method Validation Report for a 25-Marker Spectral Flow Pan-Immune Profiling Test for Human PBMCs using the Cytek® 25-Color Immunoprofiling Kit

November 2023

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1. Table of Content

2. Compliance Statement

The validation experiments were performed as per the validation study plan described in DCS127 25-Marker Spectral Flow Performance Validation Plan.docx, which is based on guidance from DCS034 Quality Assurance and Performance Verification.docx. Any deviation to this validation or any relevant SOP will be described in detail and documented as an amendment to this validation report.

3. Purpose

This document describes the performance validation data for a 25-Marker Spectral Flow Pan-Immune Profiling Test for Human Peripheral Blood Mononuclear Cells (PBMCs) using the Cytek® 25-Color Immunoprofiling Kit. This test was originally developed and validated for research use by Cytek® Biosciences, Inc. [1]. In this report, the performance of this test when performed by Teiko Bio is being evaluated. This test evaluates major human immune populations from PBMCs, including subsets in T cells, B cells, NK cells, monocytes and dendritic cells using spectral flow cytometry. The validation is designed to ensure a method developed by another manufacturer works in our hands after minor modifications, and yields immune profiling data that meets or exceeds our acceptance criteria.

4. Scope

The scope of this Fit-for-Purpose validation study is to demonstrate the robustness of the 25-Marker Spectral Flow Pan-Immune Profiling Test using the Cytek® 25-Color Immunoprofiling Kit for the measurement of 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 DCS127 25-Marker Spectral Flow Performance Validation Plan.docx and following the general guidance described in DCS034 Quality Assurance and Performance Verification.docx. 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).
In addition to method development procedures, this document describes the following performance validation results:

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

The validation parameters were assessed in PBMCs isolated from healthy human donors. The test results in this document apply to the 25-Marker Spectral Flow 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 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
PC	Percentage of change
ACD-A	Anticoagulant citrate dextrose solution formula A
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

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 the 25-Marker Spectral Flow Pan-Immune Profiling Test for

Human Peripheral Blood Mononuclear Cells (PBMCs) using the Cytek® 25-Color Immunoprofiling Kit developed by Cytek Biosciences, Inc, when performed at Teiko Bio.

8.2 Assay Design

Teiko Bio's 25-Marker Spectral Flow Pan-Immune Profiling Test for Human Peripheral Blood Mononuclear Cells (PBMCs) is a spectral flow cytometry-based test based on the Cytek® 25-Color Immunoprofiling Kit, which was designed to investigate the frequencies of major human immune subpopulations for T cells, B cells, NK cells, and myeloid cells. 25 antibodies are used to stain PBMCs, which were collected by Ficoll Density Gradient from whole blood isolated from human donors. 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. PBMCs isolated from three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006) were used for all performance tests, with the exception of accuracy which was performed using PBMCs from a single donor (PBMC-006). See Table 1 for definitions for individual cell subsets detected in this assay.

Table 1: Teiko Bio's 25-Marker Spectral Flow Pan-Immune Profiling Test cell subset definitions Total Non-Granulocytes will be defined as CD45+ Live Singlets following exclusion of Basophils (CD123+HLA-DR-) and other granulocytes (FSC-A^{hi}CD16^{hi}). All subsets will be measured as % of Total Non-Granulocytes.

Cell Subset	Definition (Marker Parameters)
Singlets	FSC-A / FSC-H
Live	ViaDye-
Total Immune Cells	CD45+
Total Non-Granulocytes	NOT(CD123+ HLA-DR-)
B cells	NOT(CD19- CD20-) CD3- gdTCR- CD14-
Naive	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD+ CD27-
Marginal Zone-like	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD+ CD27+
Memory	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD- CD27+ CD20+
Plasmablasts (PB)	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD- CD27+ CD20-
T cells	CD19- CD20- CD3+ CD14-
Gamma Delta (γδ) T cells	CD19- CD20- CD3+ CD14- gdTCR+
Natural Killer T cells (NKT)	CD19- CD20- CD3+ CD14- gdTCR- CD56+
CD4+ T cells	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8-
Regulatory T cells (Treg)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- CD25+ CD127-

nonTreg	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-)
Naive	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7+ CD45RA+
CD27+CD28+ Naive	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7+ CD45RA+ CD27+ CD28+
Central Memory (TCM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7+ CD45RA-
CD27+CD28+ TCM	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7+ CD45RA- CD27+ CD28+
Effector Memory (TEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7- CD45RA-
Early-like Effector Memory (TELEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7- CD45RA- CD27- CD28+
Early Effector Memory (TEEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7- CD45RA- CD27+ CD28+
Terminal Effector Memory (TTEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7- CD45RA- CD27- CD28-
CD45RA+ Effector Memory (TEMRA)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7- CD45RA+
CD27-CD28- TEMRA	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7- CD45RA+ CD27- CD28-
CD8+ T cells	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+
Naive	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7+ CD45RA+
CD27+CD28+ Naive	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7+ CD45RA+ CD27+ CD28+
Central Memory (TCM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7+ CD45RA-
CD27+CD28+ TCM	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7+ CD45RA- CD27+ CD28+
Effector Memory (TEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7- CD45RA-
Early-like Effector Memory (TELEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7- CD45RA- CD27- CD28+
Early Effector Memory (TEEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7- CD45RA- CD27+ CD28+

Terminal Effector Memory (TTEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7- CD45RA- CD27- CD28-
Intermediate Effector Memory (TIEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7- CD45RA- CD27+ CD28-
CD45RA+ Effector Memory (TEMRA)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7- CD45RA+
CD27-CD28- TEMRA	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CCR7- CD45RA+ CD27- CD28-
CD4/CD8 Double-Negative T cells (DNT)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8-
CD4/CD8 Double-Positive T cells (DPT)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8+
Natural Killer (NK) cells	CD19- CD20- CD3- IgD- IgM- CD14- CD56+
CD16+	CD19- CD20- CD3- IgD- IgM- CD14- CD56+ CD16+
CD16-	CD19- CD20- CD3- IgD- IgM- CD14- CD56lo CD16-
CD56hi	CD19- CD20- CD3- IgD- IgM- CD14- CD56hi CD16-
Monocytes	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ NOT(CD14- CD16-)
Classical (cMono)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14+ CD16-
Intermediate (inMono)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14+ CD16+
Non-Classical (ncMono)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16+
Dendritic Cells	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- NOT(CD11c- CD123-)
Classical (cDC)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c+ CD123-
Type 1 (cDC1)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c+ CD123- CD1C- CD141+
Type 2 (cDC2)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c+ CD123- CD1C+
Plasmacytoid (pDC)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c- CD123+
Transitional (tDC)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c+ CD123+

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.

8.3 Materials and Methods

1. Instruments
    a. Aurora 5 Laser UV/V/B/YG/R by Cytek Biosciences, Inc.
2. Software
    a. CellEngine, version June 2023 (CellCarta, Montreal, Canada)
    b. SpectroFlow, version 3.1.2 (03272023) (Cytek Biosciences Inc., Fremont, CA, USA)
    c. RStudio 4.2.3 (Posit, Boston, Massachusetts, USA)
    d. ggplot2 R package version: 3.4.2 on GitHub (San Francisco, California, USA)
3. Method
    PBMC from healthy donors were thawed, washed, counted and resuspended at a concentration of 3 million cells per mL for staining. Live samples were stained with the Cytek® 25-Color Immunoprofiling Kit and fixed with 1% PFA. Fixed samples were stored at 4°C until acquired. Major immune cell populations such as T, B, NK and myeloid cells and their corresponding subpopulations were evaluated based on the combination of fluorescent signals measured by the spectral flow cytometer.
4. Reagents

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

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
Pierce Universal Nuclease for Cell Lysis 250 KU/mL [10,000X stock]	ThermoFisher	88700
Human TruStain FcX	BioLegend	422302
RPMI 1640 Medium, with L-glutamine	Thermo Fisher	11875093

Penicillin-Streptomycin (5,000 U/mL) (100X)	Thermo Fisher	15070063
16% Paraformaldehyde Aqueous Solution (diluted to 1%)	Electron Microscopy Sciences	15710
0.5M EDTA, pH 8.0 (100X)	Thermo Fisher	15575020
30% BSA	Sigma Aldrich	A728450ML
SpectroFlo QC beads	Cytek Biosciences Inc.	B7-10001
Cytek 25-Color Immunoprofiling Assay	Cytek Biosciences Inc.	R7-40002
BioLegend Immunoprofiling Kit, 7 Color	BioLegend	900004160
Cytek FSP CompBeads	Cytek Biosciences Inc.	B7-10011
ViaDye Red Fixable Viability Dye Kit	Cytek Biosciences Inc.	R7-60008
Stain Buffer (BSA)	BD Biosciences	554657
Brilliant Stain Buffer Plus	BD Biosciences	566385
96-well plates or FACS tubes		

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 25-Marker Spectral Flow Pan-Immune Profiling test validation

No.	Target	Clone	Fluoro-chrome	Manufacturer	Catalog #	Lot #	Expiry
1	CD45RA	HI100	cFluor® V450	Cytek	R7-40002	F-022723-02	02/03/2024
2	CD20	2H7	cFluor® V547	Cytek	R7-40002	F-032823-04	02/03/2024
3	CD141	M80	cFluor® B515	Cytek	R7-40002	F-080822-04	02/03/2024
4	CD8	SK1	cFluor® B532	Cytek	R7-40002	F-121922-01	02/03/2024
5	CD14	63D3	cFluor® B548	Cytek	R7-40002	F-061623-03	02/03/2024
6	HLA-DR	L243	cFluor® B690	Cytek	R7-40002	F-031723-03	02/03/2024

7	CD25	BC96	cFluor® BYG575	Cytek	R7-40002	F-082922-02	02/03/2024
8	CD4	SK3	cFluor® YG584	Cytek	R7-40002	F-060723-04	02/03/2024
9	CD16	3G8	cFluor® BYG610	Cytek	R7-40002	F-021723-02	02/03/2024
10	IgD	IA6-2	cFluor® BYG667	Cytek	R7-40002	F-062223-01	02/03/2024
11	TCRgd	B1	cFluor® BYG710	Cytek	R7-40002	F-062623-01	02/03/2024
12	CD11c	3.9	cFluor® BYG781	Cytek	R7-40002	F-031723-05	02/03/2024
13	CD127	A019D5	cFluor® R659	Cytek	R7-40002	F-013023-01	02/03/2024
14	CD1c	L161	cFluor® R668	Cytek	R7-40002	F-010923-03	02/03/2024
15	CD19	HIB19	cFluor® R685	Cytek	R7-40002	F-062323-01	02/03/2024
16	CD123	6H6	cFluor® R720	Cytek	R7-40002	F-013123	02/03/2024
17	CD45	2D1	cFluor® R780	Cytek	R7-40002	F-020322-01	02/03/2024
18	CD27	QA17A18	cFluor® R840	Cytek	R7-40002	F-053023-01	02/03/2024
19	CD197 (CCR7)	G043H7	Brilliant Violet 421	Biolegend	900004160	B360659	12/15/2024
20	IgM	MHM-88	Brilliant Violet 510	Biolegend	900004160	B365834	10/12/2024
21	CD3	UCHT1	Brilliant Violet 570	Biolegend	900004160	B360619	10/27/2024
22	CD28	CD28.2	Brilliant Violet 650	Biolegend	900004160	B362788	12/15/2024
23	CD38	HIT2	Brilliant Violet 711	Biolegend	900004160	B360789	11/16/2024
24	CD56	5.1H11	Brilliant Violet 750	Biolegend	900004160	B362796	02/25/2024
25	CD279	EH12.2H7	Brilliant	Biolegend	900004160	B360654	11/29/2024

	(PD-1)		Violet 785				

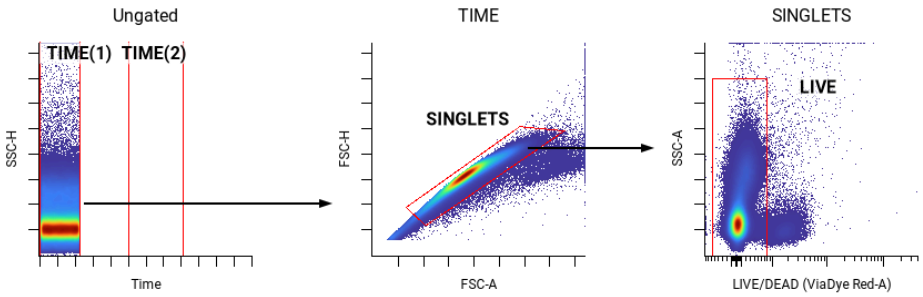
5. Sample Processing
    a. **Sample collection**
    Blood products from 3 donors (PBMC-004, PBMC-005, PBMC-006) were purchased from the Stanford Blood Bank (Palo Alto, CA, USA) as buffy coats or leukocyte reduction system (LRS) chambers. PBMCs were isolated using a Ficoll Density Gradient as described in DCS020 Isolation of PBMC by Ficoll and stored in liquid nitrogen until use.
    b. Sample preparation
    Sample preparation was performed as described in DCS125 Cytek 25-Color PBMC Immunoprofiling Assay. Briefly, cryopreserved PBMC samples were thawed and washed, then cell count and viability were assessed by Trypan Blue on a Countess for resuspension at the density of 3 million cells per mL. Next, the cells were transferred to cluster tubes for staining. Cells from each sample were put aside for single stain and unstained reference controls. The remaining sample was assessed for viability using ViaDye Red Fixable Viability Dye.
    c. Antibody staining
    Next, cells were stained with antibodies conjugated to fluorochromes using the Cytek® 25-Color Immunoprofiling Kit. Each antibody clone recognizes a specific molecule on the cell surface. Two antibodies, CCR7 and gdTCR, are added directly to the cells for 20 min prior to the addition of the remaining antibodies. The remaining antibodies are then pooled and the resulting cocktail is added to all cells at the same time to stain for an additional 20 minutes. After cell staining, the cells are washed and fixed with 1% PFA for 20 minutes. After fixation, cells are washed and resuspended for storage at 4°C. DCS125 Cytek 25-Color PBMC Immunoprofiling Assay contains more details about this part of the procedure.
    d. **Data acquisition**
    Before acquisition, samples were washed and resuspended in 300uL of PBS and acquired on an Aurora spectral flow cytometer (Cytek Biosciences, Inc.) at a rate of around 3000 events per second with abort rate less than 1%. Acquisition involves injection of the sample into the spectral flow cytometer, with cells focused into single-file as they pass through a series of lasers. These lasers excite the fluorochromes conjugated to cell-bound antibodies, and the emission spectra, along with the forward and side scatter signals, are simultaneously collected by the instrument's full range of detectors. A proprietary algorithm unmixes the spectral data to yield a signal intensity for each fluorochrome. Data output is an unmixed matrix file showing fluorescence detection signals per cell in the form of an FCS file.

e. **Data post-processing**
Manual gating of FCS files was performed using CellEngine (CellCarta, Montreal, Canada). Channel intensity was arcsinh transformed and scaled according to MFI intensity for gating. Cell populations were gated based on the parameters described in Table 1 and as shown in Figure 1. Frequencies were determined as % of total 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.

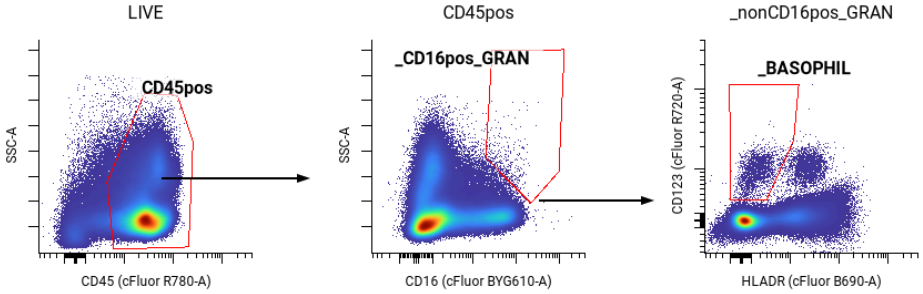
f. **Data analysis**
For precision analyses, each population as a percentage of total non-granulocytes (i.e. their frequency) was compared to its corresponding population across samples, and a coefficient of variation (CV) was calculated as a percentage: % CV = 100 * standard deviation(frequency) / mean(frequency). 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 each 24h, 48h, and 72h time point when compared to 0h was calculated as follows: % change = 100 * (frequency at 24h, 48h, or 72h - frequency at 0h) / frequency at 0h. The data were visualized with R using the ggplot2 package to display the median percent change between 0 hr and 24h, 48h, or 72h, 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 0h and 24h, 48h, 72h)/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 25-Marker Spectral Flow Pan-Immune Profiling Test

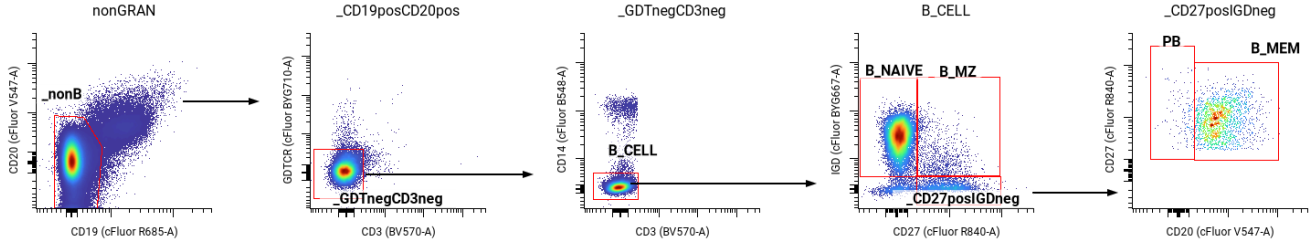
A. Gating strategy for live cells (from total events)



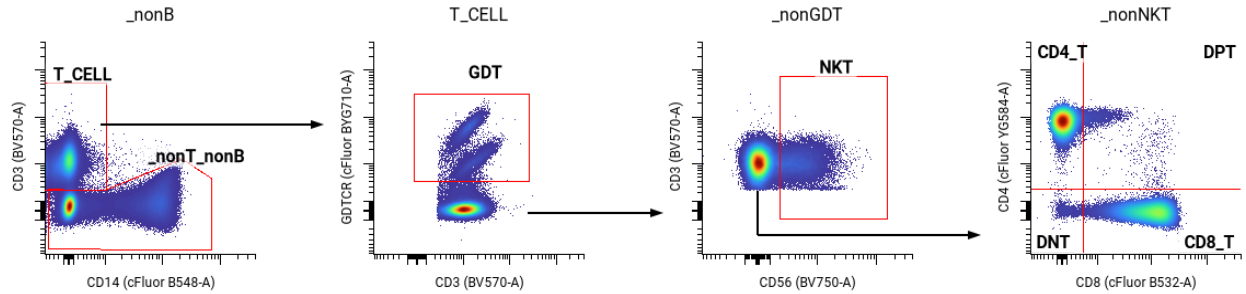
B. Gating strategy for total non-granulocytes (from live cells)



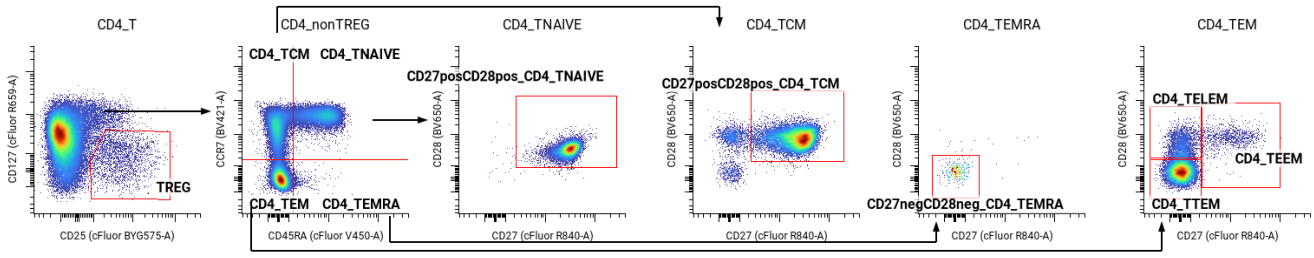
C. Gating strategy for B cells (from total non-granulocytes)



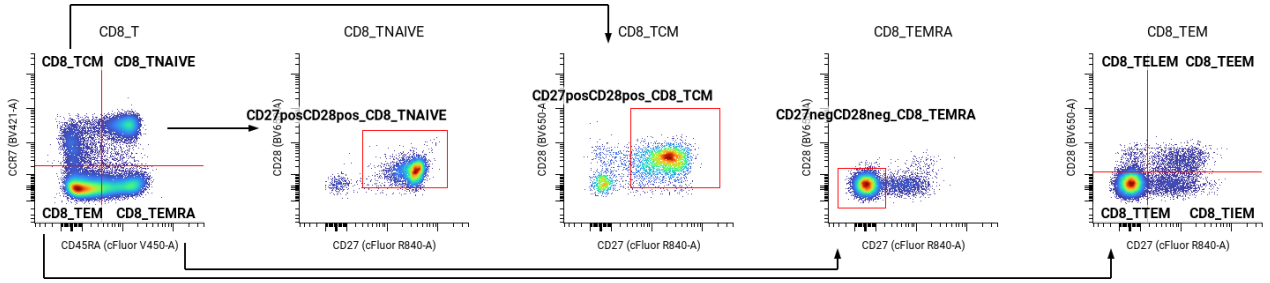
D. Gating strategy for T cells (from non-B cells)



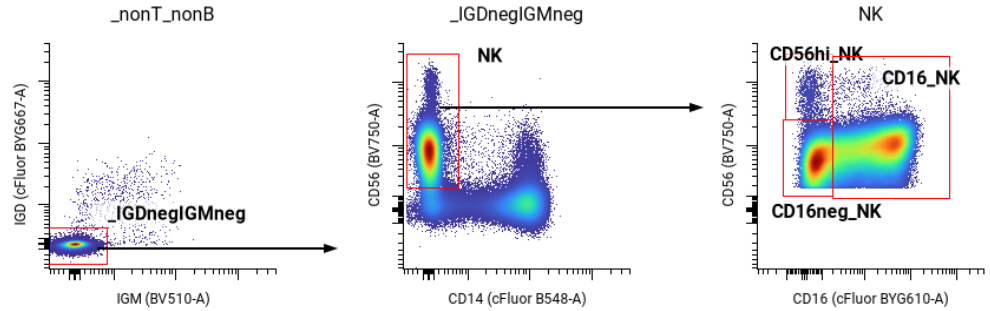
Continuation from CD4 T gate



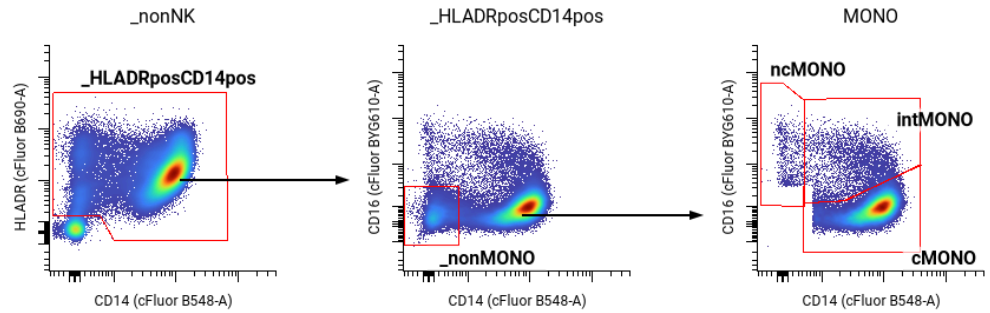
Continuation from CD8 T gate



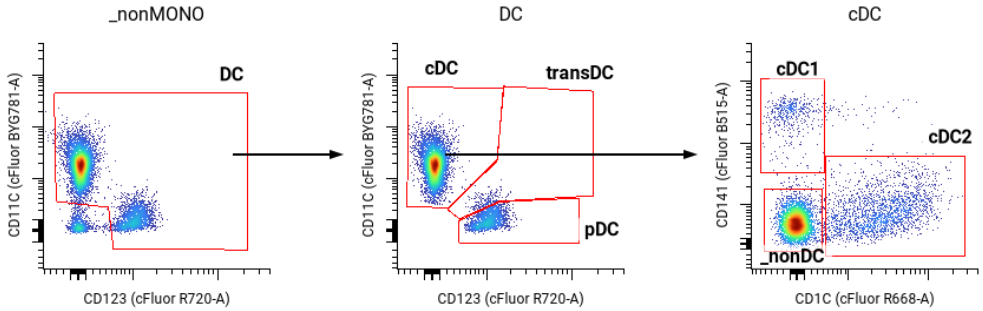
E. Gating strategy for NK cells (from non-T and non-B cells)



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



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

   Accuracy was evaluated to ensure the assay is able to measure what it proposes to measure. To ensure the positive signal for each individual immune marker derives from its antibody-fluorochrome conjugate and is not an artifact of unmixing or compensation, full-minus-multiple (FMM) controls were compared to samples stained with the full 25-color panel.

   FMM controls were designed based on the recommendations as outlined in Jensen & Wnek (2020)[2]:

   a. Nearby fluorophores (i.e. those close to each other on the emission spectrum) were distributed across FMM controls.
   b. Marker pairs used in bivariate plots were placed on different FMM controls (e.g. because CCR7 and CD45RA are used in combination to gate on T cell populations, they are used in different controls).
   c. Activation markers were distributed across FMM controls (e.g. HLA-DR, CD25).
   d. Primary lineage markers with consistently clean separation were not included in FMM controls. For the purpose of this validation plan, FMM controls were not generated for the following markers: CD45, CD3, CD4, CD8, and Viability.
   e. A FMM control was generated by combining all antibody-conjugated fluorochromes, except that of the absentee markers for each FMM, into a single cocktail.

   Accuracy was assessed by comparing frequencies of marker-positive populations in samples stained with the full 25-color panel to their corresponding FMM control. For each individual marker, biaxial plots were generated showing positive marker staining in the full panel multicolor sample and negative marker staining in the FMM for that marker. Acceptance criteria were ≤ 1% events in marker-positive gate for all 25 markers

assayed.

2. Precision
   Intra-run and Inter-run precision was evaluated to ensure test results are not affected by technical intra-run or inter-run variables.
   a. Intra-run precision was assessed by taking cryopreserved PBMCs from three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006), 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 Cytek® Aurora machine during one run. Variance between replicates was calculated for precision analysis. Acceptance criteria were CV ≤ 20% for ≥95% of all measurable (>100 cells median) immune cell populations.
   b. Inter-run precision was assessed by taking cryopreserved PBMCs from three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006) and splitting the sample into three technical replicates. Each replicate was processed (fixed, stained and run on Aurora) independently by three operators. Variance between runs was calculated for precision analysis. Acceptance criteria were CV ≤ 20% for ≥95% of all measurable (>100 cells median) immune cell populations.

3. Stability
   Post-fixation stability was evaluated to ensure test results are not affected by storage-related variables. Stability was assessed by taking cryopreserved PBMCs from three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006) and staining and fixing them by the same operator on the same day. Then, samples were split into four replicates and stored at 4°C. One set of replicates was run immediately (0 hours). The other sets of replicates were run after one day (24 hours), two days (48 hours), and three days (72 hours) of storage. 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 ≤25% change for ≥95% of all measurable (>100 cell events) immune populations from t = 0 hours.

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 three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006) and staining and fixing them by the same operator on the same day, and running on the Cytek® Aurora machine during one run. 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

   To ensure the positive signal for each individual immune marker derives from its antibody-fluorochrome conjugate and is not an artifact of unmixing or compensation, we applied this panel to a PBMC from a single donor and compared it to full-minus-multiple (FMM) controls. Table 4 shows the design for our four FMM controls.

   We were able to detect all individual markers at >1% of the total Live population in the sample stained with the full 25-marker panel. The same gates were applied to the corresponding FMM controls and the percentage of marker-positive cells within the gate was assessed. Table 5 shows the percentage of marker-positive cells for each marker in the full-panel sample and its corresponding FMM control. All markers met our acceptance criteria, with <1% marker-positive cell events in their corresponding FMM control.

Table 4. Design of full-minus-multiple (FMM) controls. We designed four FMM controls that eliminate five or six fluorochromes each.

No.	Panel	FMM1	FMM2	FMM3	FMM4
1	CD45RA	CD45RA	-	CD45RA	CD45RA
2	CD20	CD20	CD20	CD20	-
3	CD141	-	CD141	CD141	CD141
4	CD8	CD8	CD8	CD8	CD8
5	CD14	CD14	CD14	-	CD14
6	HLA-DR	HLA-DR	-	HLA-DR	HLA-DR
7	CD25	CD25	CD25	CD25	-
8	CD4	CD4	CD4	CD4	CD4
9	CD16	CD16	-	CD16	CD16
10	IgD	-	IgD	IgD	IgD
11	TCRgd	TCRgd	TCRgd	TCRgd	-
12	CD11c	CD11c	CD11c	-	CD11c
13	CD127	-	CD127	CD127	CD127
14	CD1c	CD1c	CD1c	CD1c	-
15	CD19	CD19	-	CD19	CD19
16	CD123	-	CD123	CD123	CD123

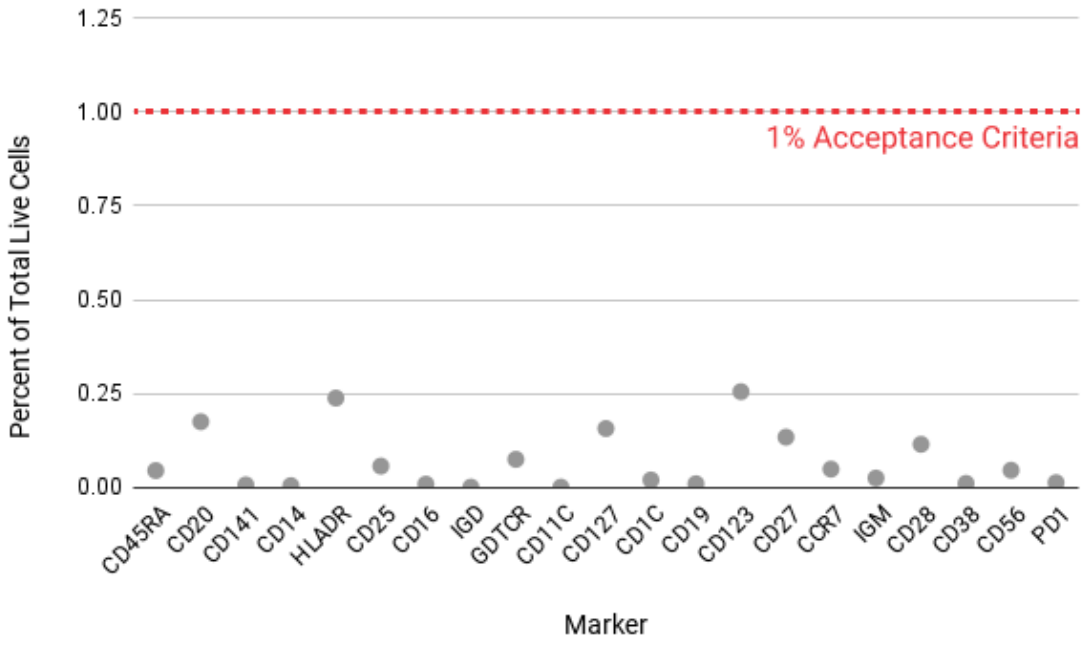
17	CD45	CD45	CD45	CD45	CD45
18	CD27	CD27	CD27	-	CD27
19	CD197 (CCR7)	-	CD197 (CCR7)	CD197 (CCR7)	CD197 (CCR7)
20	IgM	IgM	IgM	-	IgM
21	CD3	CD3	CD3	CD3	CD3
22	CD28	-	CD28	CD28	CD28
23	CD38	CD38	-	CD38	CD38
24	CD56	CD56	CD56	-	CD56
25	CD279 (PD-1)	CD279 (PD-1)	CD279 (PD-1)	CD279 (PD-1)	-

Table 5: Percentage of marker-positive cells for each marker in the full-panel sample and its corresponding FMM control. Gates were drawn for each marker within total Live cells.

No.	Marker	% Marker Positive in Full Panel	% Marker Positive in Corresponding FMM
1	CD45RA	47.75	0.045
2	CD20	6.68	0.175
3	CD141	14.95	0.007
4	CD8	N/A	N/A
5	CD14	25.25	0.005
6	HLA-DR	39.95	0.238
7	CD25	2.69	0.057
8	CD4	N/A	N/A
9	CD16	3.90	0.009
10	IgD	7.65	0.001
11	TCRgd	1.32	0.075
12	CD11c	35.54	0.001
13	CD127	28.34	0.157
14	CD1c	2.57	0.020
15	CD19	11.63	0.010
16	CD123	2.75	0.255
17	CD45	N/A	N/A
18	CD27	42.72	0.134

19	CD197 (CCR7)	74.17	0.049
20	IgM	5.12	0.025
21	CD3	N/A	N/A
22	CD28	42.64	0.115
23	CD38	60.02	0.011
24	CD56	12.68	0.046
25	CD279 (PD-1)	4.62	0.013

Figure 2: Median Frequency (Percentage of Total Non-Granulocytes) of Marker-Positive Immune Cells per Marker in its Corresponding FMM Control



2. Precision
   a. *Intra-Run*

Intra-run precision was assessed by taking cryopreserved PBMCs from three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006), 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 Cytek® Aurora instrument during one run. Variance between replicates was calculated for precision analysis and can be seen in Tables 6-8 and Figure 2. All measurable (>100 cells median) cell populations passed the acceptable threshold of ≤20% variation between replicates. **Total average intra-run %CV for all measurable populations across all three donors was 3.66%**, with individual donor average %CVs of 5.27%, 2.21%, and 3.55% for Donor 1 (PBMC-004), Donor 2 (PBMC-005), and Donor 3 (PBMC-006), respectively.

Table 6: Summary of Intra-Run Precision Assessment for Donor 1 (PBMC-004)

Cell Subset	# of Repli-cates	Median # of cells measured	Average % of non-granulocytes	Coefficient of Variation between replicates (%)
B cells	3	33121	8.48	6.89
Naive	3	27124	6.95	6.95
Marginal Zone-like	3	1809	0.45	10.81
Memory	3	2077	0.54	6.54
Plasmablasts (PB)	0*	*	*	*
T cells	3	157970	41.26	2.77
Gamma Delta (γδ) T cells	3	5050	1.31	5.24
Natural Killer T cells (NKT)	3	45462	11.93	2.85
CD4+ T cells	3	49928	12.95	3.19
Regulatory T cells (Treg)	3	3771	0.98	3.44
nonTreg	3	46157	11.97	3.17
Naive	3	9454	2.52	2.68
CD27+CD28+ Naive	3	9170	2.45	2.08
Central Memory (TCM)	3	17073	4.39	4.16
CD27+CD28+ TCM	3	15015	3.86	3.70
Effector Memory (TEM)	3	11017	2.85	3.55

Early-like Effector Memory (TELEM)	3	2124	0.55	3.08
Early Effector Memory (TEEM)	3	1732	0.45	9.41
Terminal Effector Memory (TTEM)	3	7010	1.83	2.42
CD45RA+ Effector Memory (TEMRA)	3	8613	2.22	4.68
CD27-CD28- TEMRA	3	8580	2.21	4.70
CD8+ T cells	3	53976	14.15	2.15
Naive	3	2258	0.59	7.13
CD27+CD28+ Naive	3	1802	0.47	5.14
Central Memory (TCM)	3	6136	1.54	6.36
CD27+CD28+ TCM	3	4111	1.04	4.63
Effector Memory (TEM)	3	31748	8.35	1.99
Early-like Effector Memory (TELEM)	3	676	0.17	3.87
Early Effector Memory (TEEM)	3	1529	0.38	6.15
Terminal Effector Memory (TTEM)	3	28775	7.59	1.75
Intermediate Effector Memory (TIEM)	3	768	0.21	6.00
CD45RA+ Effector Memory (TEMRA)	3	13834	3.68	5.09
CD27-CD28- TEMRA	3	13404	3.56	5.12
CD4/CD8 Double-Negative T cells (DNT)	3	857	0.21	11.38
CD4/CD8 Double-Positive T cells (DPT)	3	2697	0.71	1.60
Natural Killer (NK) cells	3	64758	17.39	2.83
CD16+	3	31436	8.48	2.96
CD16-	3	30352	8.14	2.26
CD56hi	3	2532	0.65	10.17
Monocytes	3	94005	26.32	6.47

Classical (cMono)	3	92280	25.86	6.55
Intermediate (inMono)	3	1055	0.29	3.10
Non-Classical (ncMono)	3	689	0.18	3.05
Dendritic Cells	3	11532	3.22	7.04
Classical (cDC)	3	8064	2.21	5.49
Type 1 (cDC1)	3	217	0.07	14.65
Type 2 (cDC2)	3	2309	0.69	11.48
Plasmacytoid (pDC)	3	3387	0.99	10.92
Transitional (tDC)	0*	*	*	*
Donor 1 Average				**5.27**

**Populations with a median of fewer than 100 cell events across 3 replicates were excluded from precision analysis and CV calculations. As a result, we excluded Plasmablasts and Transitional Dendritic Cells from analysis.*

Table 7: Summary of Intra-Run Precision Assessment for Donor 2 (PBMC-005)

Cell Subset	# of Repli-cates	Median # of cells measured	Average % of non-granulocytes	Coefficient of Variation between replicates (%)
B cells	3	18521	5.48	2.02
Naive	3	14356	4.27	2.61
Marginal Zone-like	3	1059	0.31	2.63
Memory	3	1855	0.54	2.30
Plasmablasts (PB)	3	148	0.04	1.12
T cells	3	129899	37.42	0.87
Gamma Delta (γδ) T cells	3	15062	4.38	1.52
Natural Killer T cells (NKT)	3	16261	4.64	2.69
CD4+ T cells	3	47269	13.64	0.59
Regulatory T cells (Treg)	3	1973	0.58	3.08
nonTreg	3	45296	13.05	0.57
Naive	3	11928	3.46	1.72
CD27+CD28+ Naive	3	11864	3.44	1.76
Central Memory (TCM)	3	15120	4.34	1.90
CD27+CD28+ TCM	3	13298	3.81	2.22

Effector Memory (TEM)	3	17705	5.16	1.13
Early-like Effector Memory (TELEM)	3	2539	0.76	3.67
Early Effector Memory (TEEM)	3	999	0.30	3.23
Terminal Effector Memory (TTEM)	3	13751	4.04	1.75
CD45RA+ Effector Memory (TEMRA)	3	310	0.09	3.09
CD27-CD28- TEMRA	3	290	0.09	3.29
CD8+ T cells	3	47544	13.77	0.61
Naive	3	6972	2.01	2.06
CD27+CD28+ Naive	3	6564	1.90	2.01
Central Memory (TCM)	3	4086	1.17	1.55
CD27+CD28+ TCM	3	2961	0.86	1.52
Effector Memory (TEM)	3	23701	6.89	0.78
Early-like Effector Memory (TELEM)	3	916	0.27	1.83
Early Effector Memory (TEEM)	3	1642	0.48	3.62
Terminal Effector Memory (TTEM)	3	19073	5.57	1.25
Intermediate Effector Memory (TIEM)	3	1990	0.58	0.26
CD45RA+ Effector Memory (TEMRA)	3	12663	3.70	1.95
CD27-CD28- TEMRA	3	11085	3.25	1.95
CD4/CD8 Double-Negative T cells (DNT)	3	1095	0.32	2.30
CD4/CD8 Double-Positive T cells (DPT)	3	2386	0.68	2.62
Natural Killer (NK) cells	3	118865	34.32	0.26
CD16+	3	76279	22.19	0.47
CD16-	3	39658	11.52	1.40
CD56hi	3	1276	0.38	3.86

Monocytes	3	56977	16.71	1.15
Classical (cMono)	3	51429	15.09	1.19
Intermediate (inMono)	3	4778	1.39	1.04
Non-Classical (ncMono)	3	795	0.24	2.71
Dendritic Cells	3	7958	2.34	2.14
Classical (cDC)	3	6156	1.79	0.61
Type 1 (cDC1)	3	391	0.11	5.60
Type 2 (cDC2)	3	1904	0.56	2.92
Plasmacytoid (pDC)	3	1639	0.48	9.09
Transitional (tDC)	3	202	0.06	7.87
Donor 2 Average				**2.21**

Table 8: Summary of Intra-Run Precision Assessment for Donor 3 (PBMC-006)

Cell Subset	# of Repli-cates	Median # of cells measured	Average % of non-granulocytes	Coefficient of Variation between replicates (%)
B cells	3	36702	8.93	0.73
Naive	3	29222	7.09	0.96
Marginal Zone-like	3	2037	0.51	1.55
Memory	3	3352	0.83	1.87
Plasmablasts (PB)	3	200	0.05	5.28
T cells	3	183098	44.67	0.22
Gamma Delta (γδ) T cells	3	7408	1.74	5.56
Natural Killer T cells (NKT)	3	4126	1.02	3.19
CD4+ T cells	3	132766	32.62	0.95
Regulatory T cells (Treg)	3	9086	2.27	3.11
nonTreg	3	123680	30.35	1.04
Naive	3	70781	17.16	0.45
CD27+CD28+ Naive	3	70613	17.12	0.46
Central Memory (TCM)	3	45251	11.20	1.84
CD27+CD28+ TCM	3	42038	10.40	1.70
Effector Memory (TEM)	3	7360	1.91	6.84

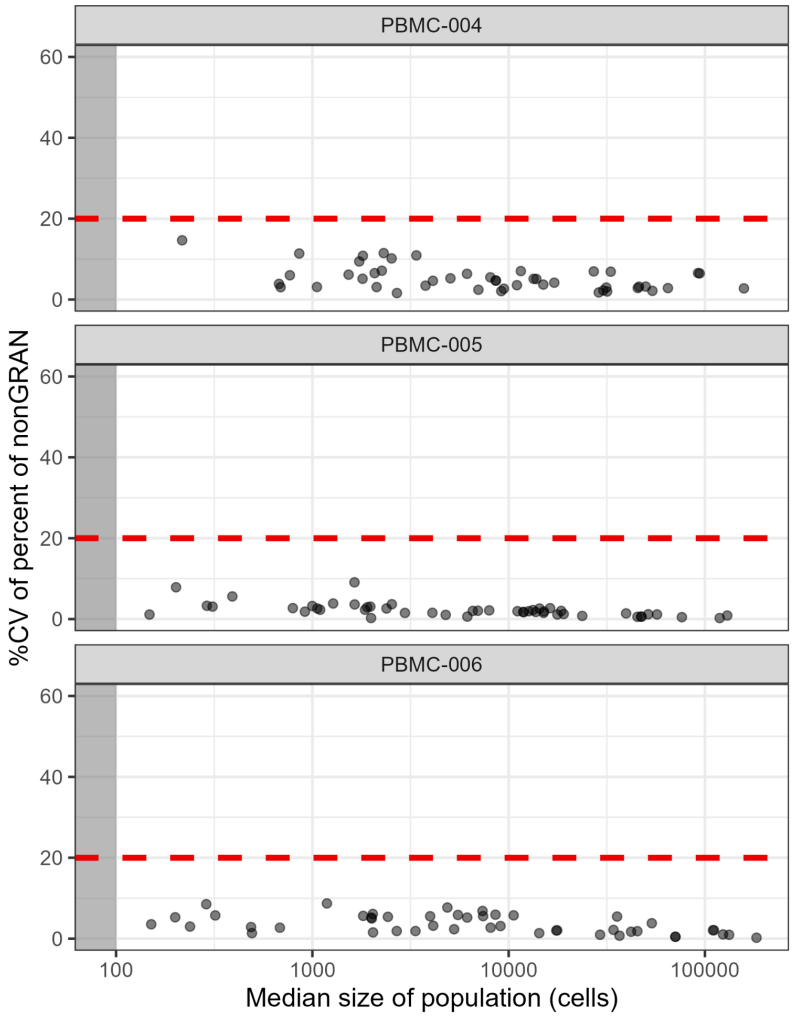
Early-like Effector Memory (TELEM)	3	2425	0.62	5.39
Early Effector Memory (TEEM)	3	4872	1.28	7.69
Terminal Effector Memory (TTEM)	0*	*	*	*
CD45RA+ Effector Memory (TEMRA)	3	288	0.08	8.51
CD27-CD28- TEMRA	0*	*	*	*
CD8+ T cells	3	34196	8.21	2.16
Naive	3	17670	4.27	2.05
CD27+CD28+ Naive	3	17483	4.23	2.01
Central Memory (TCM)	3	6147	1.44	5.21
CD27+CD28+ TCM	3	5518	1.29	5.86
Effector Memory (TEM)	3	8092	1.98	2.71
Early-like Effector Memory (TELEM)	3	683	0.17	2.69
Early Effector Memory (TEEM)	3	5274	1.31	2.33
Terminal Effector Memory (TTEM)	3	320	0.08	5.73
Intermediate Effector Memory (TIEM)	3	1815	0.43	5.62
CD45RA+ Effector Memory (TEMRA)	3	2031	0.52	6.10
CD27-CD28- TEMRA	3	492	0.12	1.36
CD4/CD8 Double-Negative T cells (DNT)	3	3981	0.95	5.53
CD4/CD8 Double-Positive T cells (DPT)	3	487	0.12	2.86
Natural Killer (NK) cells	3	53600	12.67	3.81
CD16+	3	14309	3.50	1.35
CD16-	3	35672	8.45	5.45
CD56hi	3	2690	0.65	1.88
Monocytes	3	111167	27.61	2.07

Classical (cMono)	3	109845	27.26	2.08
Intermediate (inMono)	3	1186	0.31	8.70
Non-Classical (ncMono)	3	151	0.04	3.54
Dendritic Cells	3	10601	2.71	5.75
Classical (cDC)	3	8567	2.20	5.92
Type 1 (cDC1)	3	238	0.06	2.99
Type 2 (cDC2)	3	1994	0.50	5.20
Plasmacytoid (pDC)	3	2008	0.51	4.97
Transitional (tDC)	0*	*	*	*
Donor 3 Average				**3.55**

**Populations with a median of fewer than 100 cell events across 3 replicates were excluded from precision analysis and CV calculations. As a result, we excluded CD4+ Terminal Effector Memory (TTEM) cells, CD4+ CD27-CD28- TEMRA cells, and Transitional Dendritic Cells from analysis.*

Data for individual replicates can be found in DCS133 Appendix A Teiko Bio 25-Marker Spectral Flow Pan-Immune Profiling Test Validation Report Data.

Figure 3: Coefficient of Variation in Frequency (Percentage of Total 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 three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006) and splitting the sample into three technical replicates. Each replicate was processed (fixed, stained and run on Aurora) independently by three separate analysts. Variance between runs was calculated for precision analysis and can be seen in Tables 9-11 and Figure 3. All three donors passed the acceptable threshold of CV ≤ 20% for ≥95% of all measurable (>100 cells median) immune cell populations. **Total average inter-run %CV for all measurable populations across all three donors was**

3.45%, with individual donor average %CVs of 3.26%, 3.78%, and 3.32% for Donor 1 (PBMC-004), Donor 2 (PBMC-005), and Donor 3 (PBMC-006), respectively.

Table 9: Summary of Inter-Run Precision Assessment for Donor 1 (PBMC-004)

Cell Subset	# of Repli-cates	Median # of cells measured	Average % of non-granulocytes	Coefficient of Variation between replicates (%)
B cells	3	30297	6.61	6.35
Naive	3	25503	5.58	6.87
Marginal Zone-like	3	1919	0.42	5.33
Memory	3	1682	0.37	3.77
Plasmablasts (PB)	0*	*	*	*
T cells	3	189798	42.08	1.10
Gamma Delta (γδ) T cells	3	5213	1.16	3.12
Natural Killer T cells (NKT)	3	60030	13.35	1.67
CD4+ T cells	3	57872	12.83	1.21
Regulatory T cells (Treg)	3	4340	0.96	2.03
nonTreg	3	53502	11.87	1.27
Naive	3	11709	2.61	1.20
CD27+CD28+ Naive	3	11587	2.58	1.23
Central Memory (TCM)	3	18710	4.16	2.28
CD27+CD28+ TCM	3	16510	3.65	2.05
Effector Memory (TEM)	3	11146	2.44	3.35
Early-like Effector Memory (TELEM)	3	2905	0.65	2.21
Early Effector Memory (TEEM)	3	2544	0.56	4.57
Terminal Effector Memory (TTEM)	3	5651	1.22	6.52
CD45RA+ Effector Memory (TEMRA)	3	11937	2.67	0.90
CD27-CD28- TEMRA	3	11835	2.64	0.91
CD8+ T cells	3	60925	13.46	0.56

Naive	3	3437	0.77	2.23
CD27+CD28+ Naive	3	3095	0.69	1.98
Central Memory (TCM)	3	4173	0.93	3.58
CD27+CD28+ TCM	3	3389	0.76	3.58
Effector Memory (TEM)	3	11643	2.55	5.92
Early-like Effector Memory (TELEM)	3	950	0.21	4.24
Early Effector Memory (TEEM)	3	2218	0.49	2.15
Terminal Effector Memory (TTEM)	3	8028	1.75	7.44
Intermediate Effector Memory (TIEM)	3	476	0.11	11.54
CD45RA+ Effector Memory (TEMRA)	3	41496	9.20	0.94
CD27-CD28- TEMRA	3	40106	8.88	0.88
CD4/CD8 Double-Negative T cells (DNT)	3	811	0.19	8.76
CD4/CD8 Double-Positive T cells (DPT)	3	4964	1.09	2.61
Natural Killer (NK) cells	3	76372	16.95	1.45
CD16+	3	56400	12.43	0.77
CD16-	3	17984	4.00	4.93
CD56hi	3	2061	0.46	1.64
Monocytes	3	125587	27.80	2.79
Classical (cMono)	3	120945	26.76	2.81
Intermediate (inMono)	3	3781	0.84	2.47
Non-Classical (ncMono)	3	887	0.20	3.23
Dendritic Cells	3	20700	4.51	2.49
Classical (cDC)	3	15786	3.46	2.08
Type 1 (cDC1)	3	309	0.07	8.32
Type 2 (cDC2)	3	4454	0.97	2.09
Plasmacytoid (pDC)	3	4763	1.03	3.98

Transitional (tDC)	3	137	0.03	3.01
Donor 1 Average				**3.26**

**Populations with a median of fewer than 100 cell events across 3 replicates were excluded from precision analysis and CV calculations. As a result, we excluded Plasmablasts from analysis.*

Table 10: Summary of Inter-Run Precision Assessment for Donor 2 (PBMC-005)

Cell Subset	# of Repli-cates	Median # of cells measured	Average % of non-granulocytes	Coefficient of Variation between replicates (%)
B cells	3	22750	5.46	5.81
Naive	3	18549	4.45	6.67
Marginal Zone-like	3	1368	0.32	1.15
Memory	3	1857	0.44	5.41
Plasmablasts (PB)	0*	*	*	*
T cells	3	157814	37.00	1.39
Gamma Delta (γδ) T cells	3	18377	4.30	1.37
Natural Killer T cells (NKT)	3	21231	5.04	0.87
CD4+ T cells	3	56440	13.17	2.95
Regulatory T cells (Treg)	3	2433	0.57	3.32
nonTreg	3	54007	12.59	2.94
Naive	3	15009	3.50	3.09
CD27+CD28+ Naive	3	14903	3.48	3.02
Central Memory (TCM)	3	16694	3.87	4.14
CD27+CD28+ TCM	3	14533	3.36	4.30
Effector Memory (TEM)	3	21448	5.00	2.35
Early-like Effector Memory (TELEM)	3	4045	0.95	2.66
Early Effector Memory (TEEM)	3	1395	0.33	3.64
Terminal Effector Memory (TTEM)	3	15800	3.69	2.25
CD45RA+ Effector Memory (TEMRA)	3	939	0.22	8.28

CD27-CD28- TEMRA	3	885	0.21	8.62
CD8+ T cells	3	56759	13.33	0.94
Naive	3	8353	1.95	1.46
CD27+CD28+ Naive	3	7964	1.85	1.59
Central Memory (TCM)	3	2942	0.69	4.84
CD27+CD28+ TCM	3	2453	0.57	3.77
Effector Memory (TEM)	3	12809	3.01	4.73
Early-like Effector Memory (TELEM)	3	542	0.12	4.59
Early Effector Memory (TEEM)	3	2198	0.53	5.94
Terminal Effector Memory (TTEM)	3	8623	2.01	5.36
Intermediate Effector Memory (TIEM)	3	1488	0.35	3.28
CD45RA+ Effector Memory (TEMRA)	3	32428	7.69	0.77
CD27-CD28- TEMRA	3	29004	6.86	0.43
CD4/CD8 Double-Negative T cells (DNT)	3	1281	0.30	5.99
CD4/CD8 Double-Positive T cells (DPT)	3	3631	0.86	3.62
Natural Killer (NK) cells	3	145269	33.99	0.82
CD16+	3	105854	24.94	1.17
CD16-	3	36412	8.50	3.87
CD56hi	3	1812	0.42	2.39
Monocytes	3	74534	18.07	4.29
Classical (cMono)	3	67730	16.38	3.89
Intermediate (inMono)	3	5962	1.46	8.10
Non-Classical (ncMono)	3	973	0.23	5.37
Dendritic Cells	3	13169	3.13	2.53
Classical (cDC)	3	10901	2.57	1.93
Type 1 (cDC1)	3	635	0.15	3.59

Type 2 (cDC2)	3	3276	0.77	2.61
Plasmacytoid (pDC)	3	2185	0.53	7.26
Transitional (tDC)	3	116	0.03	11.94
Donor 2 Average				**3.78**

**Populations with a median of fewer than 100 cell events across 3 replicates were excluded from precision analysis and CV calculations. As a result, we excluded Plasmablasts from analysis.*

Table 11: Summary of Inter-Run Precision Assessment for Donor 3 (PBMC-006)

Cell Subset	# of Repli-cates	Median # of cells measured	Average % of non-granulocytes	Coefficient of Variation between replicates (%)
B cells	3	40301	9.07	4.32
Naive	3	33844	7.63	4.51
Marginal Zone-like	3	2067	0.46	4.86
Memory	3	2762	0.63	4.03
Plasmablasts (PB)	0*	*	*	*
T cells	3	189090	42.36	0.96
Gamma Delta (γδ) T cells	3	6423	1.47	6.09
Natural Killer T cells (NKT)	3	4196	0.94	1.10
CD4+ T cells	3	137993	30.90	0.71
Regulatory T cells (Treg)	3	8716	1.96	1.41
nonTreg	3	129309	28.94	0.74
Naive	3	71133	16.02	0.54
CD27+CD28+ Naive	3	70855	15.95	0.58
Central Memory (TCM)	3	48406	10.86	1.56
CD27+CD28+ TCM	3	41109	9.24	1.72
Effector Memory (TEM)	3	8641	1.95	2.36
Early-like Effector Memory (TELEM)	3	3719	0.84	1.79
Early Effector Memory (TEEM)	3	4829	1.09	2.37
Terminal Effector Memory (TTEM)	0*	*	*	*

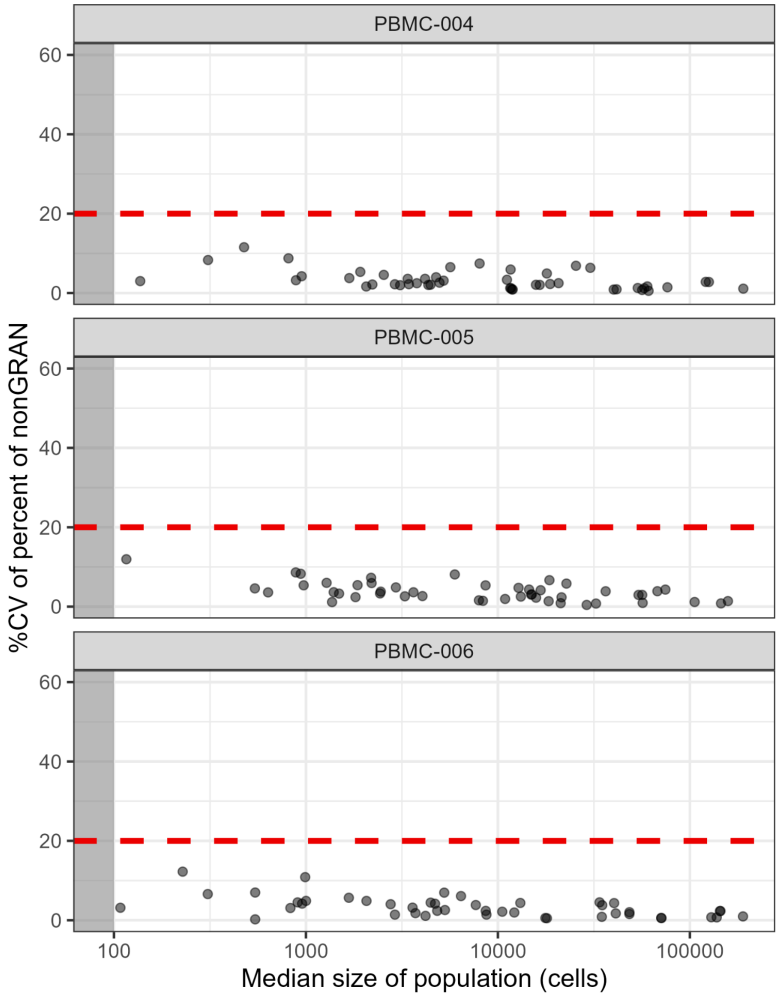
CD45RA+ Effector Memory (TEMRA)	3	544	0.12	6.98
CD27-CD28- TEMRA	0*	*	*	*
CD8+ T cells	3	34773	7.78	0.85
Naive	3	17993	4.04	0.51
CD27+CD28+ Naive	3	17667	3.97	0.56
Central Memory (TCM)	3	4464	0.98	4.44
CD27+CD28+ TCM	3	3592	0.80	3.17
Effector Memory (TEM)	3	7670	1.70	3.83
Early-like Effector Memory (TELEM)	3	1001	0.22	4.87
Early Effector Memory (TEEM)	3	5297	1.19	2.57
Terminal Effector Memory (TTEM)	3	308	0.07	6.59
Intermediate Effector Memory (TIEM)	3	991	0.22	10.86
CD45RA+ Effector Memory (TEMRA)	3	4704	1.05	4.15
CD27-CD28- TEMRA	3	955	0.21	4.18
CD4/CD8 Double-Negative T cells (DNT)	3	5252	1.14	6.95
CD4/CD8 Double-Positive T cells (DPT)	3	544	0.12	0.22
Natural Killer (NK) cells	3	48435	10.94	2.02
CD16+	3	13085	2.95	4.35
CD16-	3	34937	7.80	3.79
CD56hi	3	830	0.18	3.08
Monocytes	3	144349	32.70	2.32
Classical (cMono)	3	143357	32.46	2.31
Intermediate (inMono)	3	905	0.21	4.50
Non-Classical (ncMono)	3	108	0.02	3.14
Dendritic Cells	3	12170	2.73	1.94
Classical (cDC)	3	10532	2.34	2.15

Type 1 (cDC1)	3	228	0.05	12.24
Type 2 (cDC2)	3	2907	0.65	1.41
Plasmacytoid (pDC)	3	1673	0.38	5.66
Transitional (tDC)	0*	*	*	*
Donor 3 Average				**3.32**

**Populations with a median of fewer than 100 cell events across 3 replicates were excluded from precision analysis and CV calculations. As a result, we excluded Plasmablasts, CD4+ Terminal Effector Memory (TTEM) cells, CD4+ CD27-CD28- TEMRA cells, and Transitional Dendritic Cells from analysis.*

Data for individual replicates can be found in DCS133 Appendix A Teiko Bio 25-Marker Spectral Flow Pan-Immune Profiling Test Validation Report Data.

Figure 4: Coefficient of Variation in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Inter-Run Precision Assessment



3. Stability

   Post-fixation stability was evaluated to ensure test results are not affected by storage-related variables. Stability was assessed by taking cryopreserved PBMCs from three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006) and staining and fixing them by the same operator on the same day. Then, samples were split into four replicates and were stored at 4C. One set of replicates was run immediately (0 hours). The other sets of replicates were run after one day (24 hours), two days (48 hours), and three days (72 hours) of storage. All steps were performed by the same operator. Percentage of change in immune population frequencies was calculated independently for each time point (24 hours, 48 hours, or 72 hours) in comparison to the 0 hour time

point for stability analysis and can be seen in Tables 12-14 and Figures 4-6. **All three later timepoints met the acceptance criteria of ≤25% change for ≥95% of all measurable (>100 cell events) immune populations from t = 0 hours.** The percentage of measurable immune populations with <25% change was 98%, 96%, and 96% for 24 hours, 48 hours, and 72 hours, respectively. The measurable populations with >25% change were cDC1 at 24 hours, Treg and Intermediate Monocytes at 48 hours, and Treg and cDC1 at 72 hours.

Table 12: Summary of Stability Assessment for 24 hours post-staining and fixation

Cell Subset	# Sub-jects	Median # of cells 0h	Median # of cells 24h	Median % of Non-Granulocytes 0h	Median % of Non-Granulocytes 24h	Change between days (%)
B cells	3	57021	58083	9.43	9.44	6.59
Naive	3	48326	48538	7.65	8.03	6.70
Marginal Zone-like	3	3646	2955	0.65	0.54	-6.40
Memory	3	3401	4585	0.85	0.83	7.49
Plasmablasts (PB)	0*	*	*	*	*	*
T cells	3	242070	234099	40.57	41.07	3.71
Gamma Delta (γδ) T cells	3	9066	7595	1.73	1.69	-2.31
Natural Killer T cells (NKT)	3	16276	26792	4.96	4.58	-3.24
CD4+ T cells	3	80818	88023	15.45	15.01	4.26
Regulatory T cells (Treg)	3	7464	5968	1.09	0.86	-21.27
nonTreg	3	73354	84259	14.63	14.42	5.39
Naive	3	17866	23801	4.36	4.07	-0.48
CD27+CD28+ Naive	3	17687	23741	4.35	4.06	-0.52
Central Memory (TCM)	3	25763	29746	4.90	4.93	12.97
CD27+CD28+ TCM	3	23366	26577	4.40	4.43	12.00
Effector Memory (TEM)	3	13999	16315	2.05	2.35	14.76

Early-like Effector Memory (TELEM)	3	3225	4419	0.62	0.72	14.52
Early Effector Memory (TEEM)	3	3247	3829	0.48	0.55	16.12
Terminal Effector Memory (TTEM)	3	6863	7903	1.00	1.14	13.39
CD45RA+ Effector Memory (TEMRA)	3	725	1204	0.22	0.21	8.84
CD27-CD28- TEMRA	2	686	1152	0.21	0.20	8.64
CD8+ T cells	3	47331	85627	12.34	13.26	3.20
Naive	3	8192	13702	2.49	2.34	-2.87
CD27+CD28+ Naive	3	7984	13211	2.43	2.26	-3.04
Central Memory (TCM)	3	6009	5737	0.99	1.08	6.14
CD27+CD28+ TCM	3	5396	4747	0.83	0.87	3.78
Effector Memory (TEM)	3	11798	20873	2.97	3.01	9.08
Early-like Effector Memory (TELEM)	3	911	949	0.17	0.21	-0.18
Early Effector Memory (TEEM)	3	3661	3772	0.65	0.65	-0.24
Terminal Effector Memory (TTEM)	3	7771	15151	2.12	2.18	13.52
Intermediate Effector Memory (TIEM)	3	1214	1189	0.23	0.29	6.14
CD45RA+ Effector Memory (TEMRA)	3	24028	43289	7.32	7.41	7.97
CD27-CD28- TEMRA	3	21240	38585	6.47	6.60	8.56
CD4/CD8 Double-Negative T cells (DNT)	3	1273	1873	0.30	0.32	5.79
CD4/CD8 Double-Positive T cells	3	3467	6234	1.06	1.07	5.51

(DPT)						
Natural Killer (NK) cells	3	105993	106363	15.51	15.33	-0.43
CD16+	3	76552	83500	12.43	12.03	0.75
CD16-	3	35278	26835	6.73	6.53	-2.99
CD56hi	3	2423	2438	0.41	0.41	6.44
Monocytes	3	126866	86891	24.21	21.14	-10.18
Classical (cMono)	3	125247	85037	23.90	20.69	-10.01
Intermediate (inMono)	3	2314	2960	0.54	0.43	18.89
Non-Classical (ncMono)	3	509	884	0.15	0.15	-2.39
Dendritic Cells	3	8955	11296	1.83	1.93	-9.61
Classical (cDC)	3	7390	10127	1.66	1.73	-6.79
Type 1 (cDC1)	3	145	144	0.04	0.02	-54.27
Type 2 (cDC2)	3	2254	2727	0.43	0.47	-7.72
Plasmacytoid (pDC)	3	1539	1131	0.29	0.26	-10.42
Transitional (tDC)	1*	*	*	*	*	*
Total average						**1.61**

**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 Plasmablasts and Transitional Dendritic Cells from analysis. These populations (PB and tDC) were consistently measured at relative frequencies of <0.1% of non-granulocytes at all timepoints.*

Figure 5: Median Percentage of Change in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Stability Assessment at 24 hours compared to 0 hours

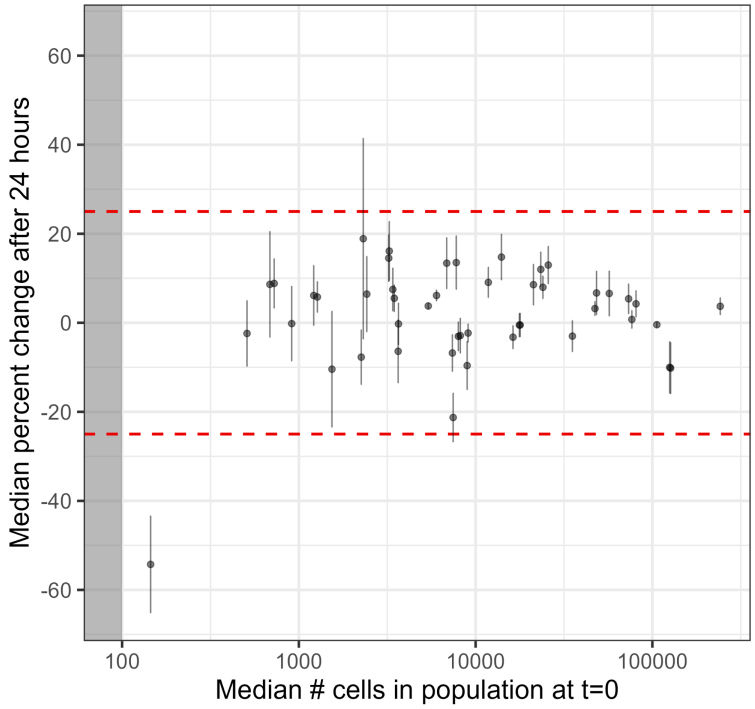


Table 13: Summary of Stability Assessment for 48 hours post-staining and fixation

Cell Subset	# Sub-jects	Median # of cells 0h	Median # of cells 48h	Median % of Non-Granulocytes 0h	Median % of Non-Granulocytes 48h	Change between days (%)
B cells	3	57021	68157	9.43	11.27	1.12
Naive	3	48326	55536	7.65	9.62	1.03
Marginal Zone-like	3	3646	4170	0.65	0.65	-8.24
Memory	3	3401	6038	0.85	0.71	0.06
Plasmablasts (PB)	0*	*	*	*	*	*
T cells	3	242070	331907	40.57	44.39	1.82
Gamma Delta (γδ) T cells	3	9066	16162	1.73	1.71	-1.24
Natural Killer T cells	3	16276	38817	4.96	4.57	-2.16

(NKT)						
CD4+ T cells	3	80818	124101	15.45	14.60	2.01
Regulatory T cells (Treg)	3	7464	5023	1.09	0.94	-29.09
nonTreg	3	73354	119509	14.63	14.06	4.64
Naive	3	17866	34020	4.36	4.00	-2.29
CD27+CD28+ Naive	3	17687	33901	4.35	3.99	-2.38
Central Memory (TCM)	3	25763	40951	4.90	4.87	13.49
CD27+CD28+ TCM	3	23366	36635	4.40	4.37	13.38
Effector Memory (TEM)	3	13999	20780	2.05	2.54	19.57
Early-like Effector Memory (TELEM)	3	3225	6585	0.62	0.72	17.19
Early Effector Memory (TEEM)	3	3247	3129	0.48	0.58	21.14
Terminal Effector Memory (TTEM)	3	6863	6907	1.00	1.29	19.12
CD45RA+ Effector Memory (TEMRA)	3	725	1995	0.22	0.23	16.55
CD27-CD28- TEMRA	2	686	1914	0.21	0.23	22.22
CD8+ T cells	3	47331	86686	12.34	14.17	1.46
Naive	3	8192	19410	2.49	2.28	-4.71
CD27+CD28+ Naive	3	7984	18708	2.43	2.20	-4.76
Central Memory (TCM)	3	6009	7959	0.99	1.18	2.73
CD27+CD28+ TCM	3	5396	6538	0.83	0.99	0.84
Effector Memory (TEM)	3	11798	21507	2.97	3.47	10.61
Early-like Effector Memory (TELEM)	3	911	1455	0.17	0.22	17.88

Early Effector Memory (TEEM)	3	3661	5493	0.65	0.65	7.27
Terminal Effector Memory (TTEM)	3	7771	13493	2.12	2.41	18.93
Intermediate Effector Memory (TIEM)	3	1214	2480	0.23	0.26	13.17
CD45RA+ Effector Memory (TEMRA)	3	24028	47434	7.32	7.32	9.10
CD27-CD28- TEMRA	3	21240	45413	6.47	6.52	14.84
CD4/CD8 Double-Negative T cells (DNT)	3	1273	2520	0.30	0.30	8.35
CD4/CD8 Double-Positive T cells (DPT)	3	3467	6603	1.06	1.06	14.02
Natural Killer (NK) cells	3	105993	114597	15.51	16.87	-2.86
CD16+	3	76552	69545	12.43	12.98	-0.80
CD16-	3	35278	59182	6.73	6.26	3.99
CD56hi	3	2423	3037	0.41	0.46	11.90
Monocytes	3	126866	120428	24.21	22.48	-3.53
Classical (cMono)	3	125247	117312	23.90	21.90	-4.13
Intermediate (inMono)	3	2314	3595	0.54	0.38	33.66
Non-Classical (ncMono)	3	509	1211	0.15	0.15	-2.53
Dendritic Cells	3	8955	14961	1.83	2.28	-7.45
Classical (cDC)	3	7390	12464	1.66	1.87	-6.56
Type 1 (cDC1)	3	145	243	0.04	0.03	-7.16
Type 2 (cDC2)	3	2254	4594	0.43	0.49	12.91
Plasmacytoid (pDC)	3	1539	2462	0.29	0.30	-11.38
Transitional (tDC)	1*	*	*	*	*	*
Total average						**4.97**

**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 Plasmablasts and Transitional Dendritic Cells from analysis. These populations (PB and tDC) were consistently measured at relative frequencies of <0.1% of non-granulocytes at all timepoints.

Figure 6: Median Percentage of Change in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Stability Assessment at 48 hours compared to 0 hours

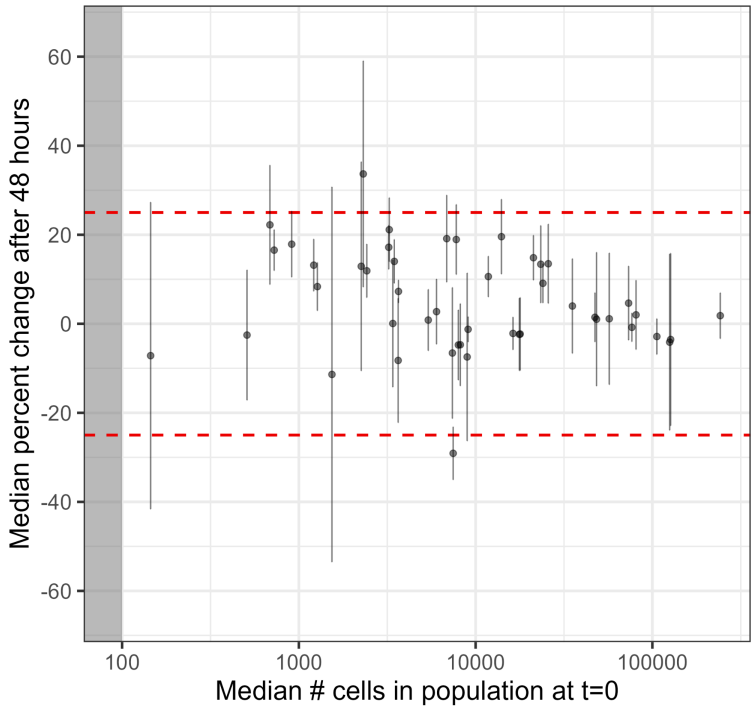


Table 14: Summary of Stability Assessment for 72 hours post-staining and fixation

Cell Subset	# Sub-jects	Median # of cells 0h	Median # of cells 72h	Median % of Non-Granulocytes 0h	Median % of Non-Granulocytes 72h	Change between days (%)
B cells	3	57021	91138	9.43	11.45	1.72
Naive	3	48326	73699	7.65	9.74	0.87
Marginal Zone-like	3	3646	4960	0.65	0.63	-15.83
Memory	3	3401	8144	0.85	0.69	2.62
Plasmablasts (PB)	0*	*	*	*	*	*

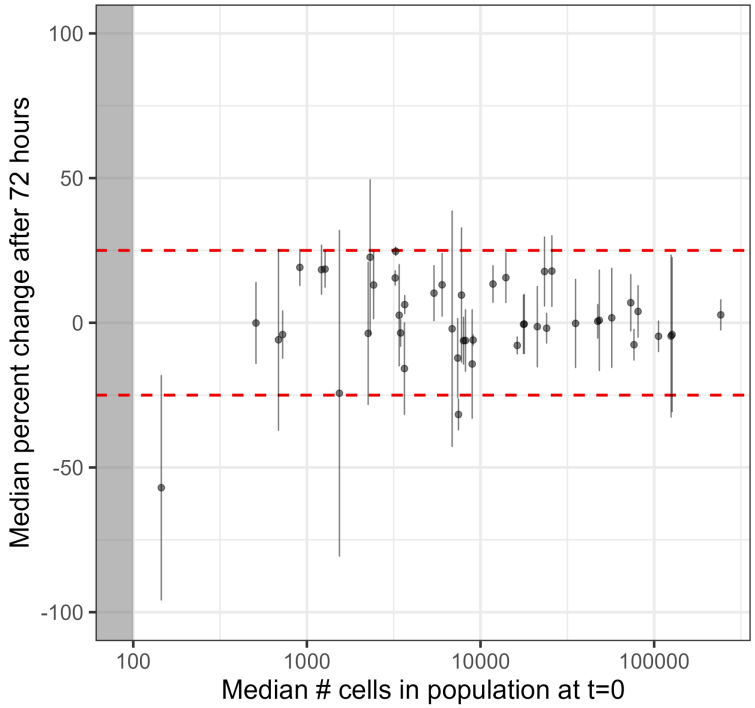
T cells	3	242070	374911	40.57	43.67	2.74
Gamma Delta (γδ) T cells	3	9066	12852	1.73	1.63	-5.96
Natural Killer T cells (NKT)	3	16276	56606	4.96	4.57	-7.82
CD4+ T cells	3	80818	172263	15.45	14.32	3.92
Regulatory T cells (Treg)	3	7464	5617	1.09	0.81	-31.69
nonTreg	3	73354	166646	14.63	13.51	6.94
Naive	3	17866	44518	4.36	3.59	-0.42
CD27+CD28+ Naive	3	17687	44359	4.35	3.58	-0.53
Central Memory (TCM)	3	25763	54756	4.90	4.98	17.87
CD27+CD28+ TCM	3	23366	49254	4.40	4.48	17.72
Effector Memory (TEM)	3	13999	16778	2.05	2.69	15.60
Early-like Effector Memory (TELEM)	3	3225	5615	0.62	0.71	15.50
Early Effector Memory (TEEM)	3	3247	5293	0.48	0.60	24.71
Terminal Effector Memory (TTEM)	3	6863	7731	1.00	1.42	-2.08
CD45RA+ Effector Memory (TEMRA)	3	725	2564	0.22	0.21	-4.08
CD27-CD28- TEMRA	2	686	2436	0.21	0.20	-5.88
CD8+ T cells	3	47331	78125	12.34	13.92	0.51
Naive	3	8192	25653	2.49	2.07	-6.13
CD27+CD28+ Naive	3	7984	24751	2.43	2.00	-6.17
Central Memory (TCM)	3	6009	10247	0.99	1.27	13.12
CD27+CD28+ TCM	3	5396	8739	0.83	1.01	10.24

Effector Memory (TEM)	3	11798	20771	2.97	3.81	13.41
Early-like Effector Memory (TELEM)	3	911	1665	0.17	0.21	19.14
Early Effector Memory (TEEM)	3	3661	8128	0.65	0.66	6.27
Terminal Effector Memory (TTEM)	3	7771	15608	2.12	2.59	9.58
Intermediate Effector Memory (TIEM)	3	1214	2352	0.23	0.30	18.35
CD45RA+ Effector Memory (TEMRA)	3	24028	45917	7.32	7.18	-1.88
CD27-CD28- TEMRA	3	21240	44251	6.47	6.38	-1.34
CD4/CD8 Double-Negative T cells (DNT)	3	1273	3762	0.30	0.30	18.55
CD4/CD8 Double-Positive T cells (DPT)	3	3467	6458	1.06	1.00	-3.52
Natural Killer (NK) cells	3	105993	90992	15.51	16.71	-4.66
CD16+	3	76552	68307	12.43	12.54	-7.58
CD16-	3	35278	48917	6.73	6.19	-0.21
CD56hi	3	2423	3735	0.41	0.47	13.06
Monocytes	3	126866	180431	24.21	22.39	-4.07
Classical (cMono)	3	125247	167911	23.90	21.90	-4.58
Intermediate (inMono)	3	2314	2852	0.54	0.36	22.67
Non-Classical (ncMono)	3	509	1071	0.15	0.16	-0.07
Dendritic Cells	3	8955	13229	1.83	2.43	-14.23
Classical (cDC)	3	7390	10744	1.66	1.97	-12.19
Type 1 (cDC1)	3	145	94	0.04	0.01	-56.99
Type 2 (cDC2)	3	2254	3274	0.43	0.50	-3.64

Plasmacytoid (pDC)	3	1539	2460	0.29	0.37	-24.35
Transitional (tDC)	1*	*	*	*	*	*
Total average						**0.62**

**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 Plasmablasts and Transitional Dendritic Cells from analysis. These populations (PB and tDC) were consistently measured at relative frequencies of <0.1% of non-granulocytes at all timepoints.*

Figure 7: Median Percentage of Change in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Stability Assessment at 72 hours compared to 0 hours



Data for individual replicates can be found in DCS133 Appendix A Teiko Bio 25-Marker Spectral Flow Pan-Immune Profiling Test Validation Report Data.

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 three (3) healthy donors (PBMC-004, PBMC-005, PBMC-006) and staining and fixing them by the same operator on the same day, and running on the Cytek® Aurora machine during one run. 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 15.

Table 15: Reference Range Values for PBMCs from Healthy Subjects

		% of Non-Granulocytes		
Cell Subset	**# of Subjects**	**Median**	**Standard deviation**	**Range**
B cells	3	6.64	1.87	5.24 - 8.94
Naive	3	5.58	1.66	4.20 - 7.50
Marginal Zone-like	3	0.45	0.08	0.33 - 0.49
Memory	3	0.41	0.13	0.37 - 0.62
Plasmablasts (PB)	0*	*	*	*
T cells	3	41.54	3.07	36.42 - 41.93
Gamma Delta (γδ) T cells	3	1.40	1.73	1.12 - 4.24
Natural Killer T cells (NKT)	3	5.09	6.21	0.93 - 13.14
CD4+ T cells	3	12.72	10.41	12.67 - 30.72
Regulatory T cells (Treg)	3	0.96	0.71	0.55 - 1.93
nonTreg	3	12.17	9.73	11.70 - 28.78
Naive	3	3.38	7.55	2.63 - 16.07
CD27+CD28+ Naive	3	3.36	7.53	2.60 - 16.00
Central Memory (TCM)	3	4.05	3.94	3.69 - 10.69
CD27+CD28+ TCM	3	3.57	3.30	3.19 - 9.08
Effector Memory (TEM)	3	2.34	1.60	1.90 - 4.87
Early-like Effector Memory (TELEM)	3	0.82	0.14	0.64 - 0.92
Early Effector Memory (TEEM)	3	0.57	0.38	0.32 - 1.07
Terminal Effector Memory (TTEM)	2*	*	*	*
CD45RA+ Effector	3	0.24	1.45	0.12 - 2.68

Memory (TEMRA)				
CD27-CD28- TEMRA	2*	*	*	*
CD8+ T cells	3	13.20	3.22	7.71 - 13.38
Naive	3	1.93	1.66	0.79 - 4.06
CD27+CD28+ Naive	3	1.84	1.67	0.71 - 4.00
Central Memory (TCM)	3	0.90	0.15	0.65 - 0.93
CD27+CD28+ TCM	3	0.74	0.12	0.55 - 0.77
Effector Memory (TEM)	3	2.39	0.62	1.62 - 2.86
Early-like Effector Memory (TELEM)	3	0.20	0.04	0.13 - 0.21
Early Effector Memory (TEEM)	3	0.51	0.38	0.49 - 1.16
Terminal Effector Memory (TTEM)	3	1.60	0.98	0.06 - 1.89
Intermediate Effector Memory (TIEM)	3	0.19	0.12	0.10 - 0.34
CD45RA+ Effector Memory (TEMRA)	3	7.77	4.36	1.09 - 9.30
CD27-CD28- TEMRA	3	6.89	4.57	0.21 - 8.97
CD4/CD8 Double-Negative T cells (DNT)	3	0.28	0.48	0.18 - 1.05
CD4/CD8 Double-Positive T cells (DPT)	3	0.89	0.50	0.12 - 1.06
Natural Killer (NK) cells	3	16.67	12.02	10.74 - 33.87
CD16+	3	12.34	11.11	3.08 - 25.20
CD16-	3	7.47	2.32	3.82 - 8.12
CD56hi	3	0.42	0.15	0.18 - 0.45
Monocytes	3	28.67	7.41	18.97 - 33.52
Classical (cMono)	3	27.52	8.20	17.10 - 33.27
Intermediate (inMono)	3	0.88	0.70	0.22 - 1.61
Non-Classical (ncMono)	3	0.26	0.14	0.03 - 0.27
Dendritic Cells	3	3.19	0.94	2.77 - 4.57
Classical (cDC)	3	2.59	0.61	2.35 - 3.50

Type 1 (cDC1)	3	0.06	0.05	0.05 - 0.15
Type 2 (cDC2)	3	0.77	0.16	0.66 - 0.98
Plasmacytoid (pDC)	3	0.58	0.33	0.40 - 1.04
Transitional (tDC)	1*	*	*	*

**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 reference range calculations. As a result, we excluded Plasmablasts, CD4+ Terminal Effector Memory (TTEM), CD4+ CD27-CD28- TEMRA, and Transitional Dendritic Cells from reference range calculations.*

Data for individual replicates can be found in DCS133 Appendix A Teiko Bio 25-Marker Spectral Flow Pan-Immune Profiling Test Validation Report Data.

8.6 Summary

Teiko Bio's 25-Marker Spectral Flow Pan-Immune Profiling Test was built on the Cytek® 25-Color Immunoprofiling Kit and validated on human PBMCs. 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 accuracy, precision within runs (intra-run), and precision between runs (inter-run) were met for all reportable cell populations. In addition, the acceptance criteria for stability up to 72 hours were met for all reportable cell populations for stained and fixed samples stored at 4C.

9. References

Appendices

- DCS133 Appendix A Teiko Bio 25-Marker Spectral Flow Pan-Immune Profiling Test Validation Report Data

Standard Operating Procedures

- DCS020 Isolation of PBMC by Ficoll
- DCS034 Quality Assurance and Performance Verification
- DCS124 Cytek® Aurora Operations and Maintenance
- DCS125 Cytek® 25-Color PBMC Immunoprofiling assay
- DCS126 Cytek® data acquisition on SpectraFlo

Research Articles

1. Cytek Biosciences Cytek® 25-Color Immunoprofiling Assay Validation Report. DOC-000327, Rev. B. Cytek Biosciences, Inc. Effective Date 17 AUG 2022. Accessed Aug 2023.

https://welcome.cytekbio.com/hubfs/DOC-00327%20Rev.%20B_Cytek%2025%20Color%20IP%20kit_Validation%20Report.pdf

2. Jensen HA, Wnek R. Analytical performance of a 25-marker spectral cytometry immune monitoring assay in peripheral blood.Cytometry. 2021;99:180–193. https://doi.org/10.1002/cyto.a.24290

10. History block

Revision	Originator	Date Effective	Nature of Change
01	Carlos Medina	11/01/2023	Initial release

Reviewed by

Name	Date	Signature
Li-Chun Cheng	11/01/2023	
Jacek Klepacki	11/03/2023