



Method Validation Report for a 25-Marker Spectral
Flow Pan-Immune Profiling Test for Human Whole
Blood Collected Using the Sodium Heparin
Stable-Lyse Stable-Store TokuKit

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2. Compliance Statement

The validation experiments were performed as per the validation study plan described in DCS148 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 Whole Blood (WB) collected Teiko's TokuKit, using sodium heparin as an anticoagulant and Stable-Lyse, Stable-Store (SmartTube Inc.) as cell fixative. This test was adapted from a 25-marker Human Peripheral Blood Mononuclear Cells (PBMC) Pan-Immunoprofiling kit validated for research use by Cytex® Biosciences, Inc. [1]. Changes made to the panel developed by Cytex® Biosciences are outlined within this report. In this report, the performance of this test when performed by Teiko is being evaluated. This test evaluates major human immune populations from whole blood, including subsets in T cells, B cells, NK cells, monocytes and dendritic cells using spectral flow cytometry. 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 Teiko's 25-Marker WB Spectral Flow Pan-Immune Profiling Test for the measurement of immune subsets from whole blood collected from healthy human clinical specimens. The method follows the general development and validation procedure described in DCS148 25-Marker Spectral Flow 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).

In addition to method development procedures, this document describes the following performance validation results:

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

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The validation parameters were assessed in whole blood from healthy human donors collected into sodium heparin vacutainers and stabilized using the TokuKit's Stable Lyse Stable Store buffer system. The test results in this document apply to the 25-Marker WB Spectral Flow Pan-Immune Profiling Test provided by Teiko Bio located at 675 S Arapen Dr. Suite 301, Salt Lake City, Utah 84108.

5. Responsibility

The Laboratory Director of the laboratory and the 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
WB	Whole Blood
CV	Coefficient of variation
PC	Percentage of change
PBS	Phosphate-buffered saline
RT	Room temperature
PFA	Paraformaldehyde
CSB	Cell staining buffer
BSA	Bovine serum albumin
FCS	Flow cytometry standard
SOP	Standard operating procedures

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7. Definitions

Term	Definition
Performance validation	Any systematic process of checking equipment against predefined process requirements, ensuring continuous operation within specifications and compliance with quality standards
Accuracy	Verification that the assay is able to measure what it proposes to measure
Precision	Closeness of agreement between independent test results obtained under stipulated conditions; reproducibility
Limit of detection	The lowest amount of analyte in a sample that can be consistently and reliably detected
Limit of blank	The highest signal observed when no analyte is present.
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's 25-Marker WB Spectral Flow Pan-Immune Profiling Test for Human Whole Blood.

8.2 Assay Design

Teiko's 25-Marker WB Spectral Flow Pan-Immune Profiling Test for Human Whole Blood is a spectral flow cytometry-based test 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 whole blood from human donors, collected with Teiko's TokuKit. In this report, the performance of the panel is described for different validation parameters: accuracy, precision,

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and stability. We also set a reference range for healthy subjects. Performance tests were assessed using three (3) whole blood specimens collected from six (6) healthy donors (WB-011, WB-012, WB-013, WB-030, WB-031, WB-032), with the exception of accuracy which was performed using whole blood from a single donor (WB-011). See Table 1 for definitions for individual cell subsets detected in this assay.

Phenotypic populations

Table 1: Teiko Bio's 25-Marker Spectral Flow Pan-Immune Profiling Test cell subset definitions and top level gates

Cell Subset	Definition (Marker Parameters)	Top level gate
Singlets	F SC-A / FSC-H	
Total Leukocytes	CD45+	
Neutrophils	CD66b+	Total Leukocytes
Basophils	CD123+ HLA-DR-)	Total Leukocytes
Total Non-Granulocytes	CD66b- AND NOT(CD123+ HLA-DR-)	
B cells	NOT(CD19- CD20-) CD3- gdTCR- CD14-	Total Non-Gran
Memory	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD- CD27+ CD20+	Total Non-Gran
Switched	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD- CD27+ CD20+ IgM-	Total Non-Gran
Unswitched	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD- CD27+ CD20+ IgM+	Total Non-Gran
Marginal Zone-like	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD+ CD27+	Total Non-Gran
Naive	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD+ CD27-	Total Non-Gran
Plasmablasts (PB)	NOT(CD19- CD20-) CD3- gdTCR- CD14- IgD- CD27+ CD20-	Total Non-Gran
T cells	CD19- CD20- CD3+ CD14-	Total Non-Gran
Gamma Delta ($\gamma\delta$) T cells	CD19- CD20- CD3+ CD14- gdTCR+	Total Non-Gran
Natural Killer T cells (NKT)	CD19- CD20- CD3+ CD14- gdTCR- CD56+	Total Non-Gran
CD4+ T cells	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8-	Total Non-Gran

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Regulatory T cells (Treg)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- CD25+ CD127-	Total Non-Gran
nonTreg	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-)	Total Non-Gran
Naive	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CD27+ CD45RA+	Total Non-Gran
Central Memory (TCM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CD27+ CD45RA-	Total Non-Gran
CD27+CD28+ TCM	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CCR7+ CD45RA- CD27+ CD28+	Total Non-Gran
Effector Memory (TEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CD27- CD45RA-	Total Non-Gran
CD45RA+ Effector Memory (TEMRA)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8- NOT(CD25+ CD127-) CD27- CD45RA+	Total Non-Gran
CD8+ T cells	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+	Total Non-Gran
Naive	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CD27+ CD45RA+	Total Non-Gran
Central Memory (TCM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CD27+ CD45RA-	Total Non-Gran
Effector Memory (TEM)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CD27- CD45RA-	Total Non-Gran
CD45RA+ Effector Memory (TEMRA)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8+ CD27- CD45RA+	Total Non-Gran
CD4/CD8 Double-Negative T cells (DNT)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4- CD8-	Total Non-Gran
CD4/CD8 Double-Positive T cells (DPT)	CD19- CD20- CD3+ CD14- gdTCR- CD56- CD4+ CD8+	Total Non-Gran
Natural Killer (NK) cells	CD19- CD20- CD3- IgD- IgM- CD14- CD56+	Total Non-Gran
CD16+	CD19- CD20- CD3- IgD- IgM- CD14- CD56+ CD16+	Total Non-Gran
CD16-	CD19- CD20- CD3- IgD- IgM- CD14- CD56lo CD16-	Total Non-Gran
CD56hi	CD19- CD20- CD3- IgD- IgM- CD14- CD56hi CD16-	Total Non-Gran
Monocytes	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+	Total Non-Gran

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	NOT(CD14- CD16-)	
Classical (cMono)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14+ CD16-	Total Non-Gran
Intermediate (inMono)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14+ CD16+	Total Non-Gran
Non-Classical (ncMono)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16+	Total Non-Gran
Dendritic Cells	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- NOT(CD11c- CD123-)	Total Non-Gran
Classical (cDC)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c+ CD123-	Total Non-Gran
Type 1 (cDC1)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c+ CD123- CD1c- CD141+	Total Non-Gran
Type 2 (cDC2)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c+ CD123- CD1c+	Total Non-Gran
Plasmacytoid (pDC)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c- CD123+	Total Non-Gran
Transitional (tDC)	CD19- CD20- CD3- IgD- IgM- CD56- HLADR+ CD14- CD16- CD11c+ CD123+	Total Non-Gran

Note: Neutrophils and Basophils will be measured as % of Total Leukocytes. All other subsets will be measured as % of Total Non-Granulocytes. Total Non-Granulocytes will be defined as CD45+ Live Singlets following exclusion of Neutrophils (CD66b+), Basophils (CD123+HLA-DR-) and other granulocytes (FSC-A^{hi}CD16^{hi}). 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.

Functional marker subsets

Functional marker frequency is calculated by dividing the number of events in the marker-positive gate by the total events in the parent (cell population) gate, with the result reported as a percentage of the parent population. Each gated population is represented as “**Parent Population > Functional Marker Subset**”. For example, CD25 expressing B cells is labeled as B_CELL > CD25 in the analysis. See Table 2 for the list of functional markers assessed in each immune cell population.

Table 2: Functional Marker Assessed in Immune Cell Populations

Functional State Marker	T cells and subsets	B cells and subsets	NK cells and subsets	DC and subsets	Monocytes and subsets	Granulocytes
CD8			gated			
CD25		gated				

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CD28	gated					
CD38	gated		gated			
HLADR	gated	gated	gated			gated
PD1	gated	gated	gated	gated	gated	

8.3 Materials and Methods

1. Instruments

- Aurora 5 Laser UV/V/B/YG/R by Cytek Biosciences, Inc.

2. Software

- CellEngine, version June 2023 (CellCarta, Montreal, Canada)
- SpectroFlow, version 3.1.2 (03272023) (Cytek Biosciences Inc., Fremont, CA, USA)

3. Method

TokuKit collected whole blood was thawed at 37 C, washed, counted and resuspended at a concentration of 3 million cells per 100uL for staining. Samples were stained with Teiko's 25-Marker WB Spectral Flow Pan-Immune Profiling Test. 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 3: List of critical reagents to be used for validation

Material	Manufacturer	Catalog #
Trypan Blue Dye 0.4% [2X stock]	Thermo Scientific	T10282
Countess™ Cell Counting Slide Chamber	Thermo Scientific	C10228
1X PBS	Standard BioTools	201058
Human TruStain FcX	BioLegend	422302
10X eBioscience permeabilization buffer	Thermo Fisher	00-8333-56
SpectroFlo QC beads	Cytek Biosciences Inc.	B7-10001
Cytek FSP CompBeads	Cytek Biosciences Inc.	B7-10011
Stain Buffer (BSA)	BD Biosciences	554657
Brilliant Stain Buffer Plus	BD Biosciences	566385

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96-well plates or FACS tubes		
Fluorochrome-labeled antibodies	various, refer to Table 3	

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

Table 4. List of antibodies used for Teiko's 25-Marker WB Spectral Flow Pan-Immune Profiling test validation

No.	Target	Clone	Fluoro- chrome	Manufacturer	Catalog #	Lot #	Expiry
1	CD66b	G10F5	Spark UV™ 387	Biolegend	305128	B420943	12/05/2028
2	CD25	M-A251	BUV™ 661	BD Biosciences	741608	4302059	10/31/2025
3	CD127	eBioRDR5	BUV™ 737	Thermo Fisher	367-1278-4 2	2867089	04/30/2028
4	CD45RA	HI100	cFluor® V450	Cytek	R7-20122	F-110223-03	05/12/2025
5	CD20	2H7	cFluor® V547	Cytek	R7-20112	C020903052 4R72	05/12/2025
6	CD141	M80	cFluor® B515	Cytek	R7-20114	F-062323-03	05/12/2025
7	CD8	SK1	cFluor® B532	Cytek	R7-20124	F-051223-01	05/12/2025
8	CD14	63D3	cFluor® B548	Cytek	R7-20116	F-121823-01	05/12/2025
9	HLA-DR	L243	cFluor® B690	Cytek	R7-20126	C995205082 4R72	05/12/2025
10	CD4	SK3	cFluor® YG584	Cytek	R7-20042	F-091323-03	05/12/2025
11	CD16	3G8	cFluor® BYG610	Cytek	R7-20130	F-091423-03	05/12/2025
12	IgD	IA6-2	cFluor® BYG667	Cytek	R7-20138	F-062223-01	05/12/2025
13	TCRgd	B1	cFluor® BYG710	Cytek	R7-20136	F-121523-02	05/12/2025
14	CD11c	3.9	cFluor® BYG781	Cytek	R7-20132	F-070623-04	05/12/2025
15	CD1c	L161	cFluor®	Cytek	R7-20120	F-070623-05	05/12/2025

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			R668				
16	CD19	HIB19	cFluor® R685	Cytek	R7-20118	F-062323-01	05/12/2025
17	CD123	6H6	cFluor® R720	Cytek	R7-20014	F-092123-01	05/12/2025
18	CD45	2D1	cFluor® R780	Cytek	R7-20134	F-091823-05	05/12/2025
19	CD27	QA17A18	cFluor® R840	Cytek	R7-20082	C027805082 4R72	05/12/2025
20	IgM	MHM-88	Brilliant Violet 510	Biolegend	314522	B425222	10/31/2025
21	CD3	UCHT1	Brilliant Violet 570	Biolegend	300436	B411287	04/03/2026
22	CD28	CD28.2	Brilliant Violet 650	Biolegend	302946	B421901	12/19/2025
23	CD38	HIT2	Brilliant Violet 711	Biolegend	303528	B399734	11/02/2025
24	CD56	5.1H11	Brilliant Violet 750	Biolegend	362556	B406332	05/17/2026
25	CD279 (PD-1)	EH12.2H7	Brilliant Violet 785	Biolegend	329930	B410666	02/27/2026

5. Sample Processing

a. **Sample collection**

Whole blood was collected from healthy volunteers into sodium heparin vacutainers. Blood was processed with the TokuKit's reagents as described in DCS138 and stored at -80C until use.

b. **Sample preparation**

Sample preparation was performed as described in DCS138 Cytex 25-Color Whole Blood Immunoprofiling Assay. Briefly, stabilized whole blood samples were thawed and washed, counted on a Countess cell counter. 3 million of cells were transferred into a cluster tube for multicolor staining. 0.3 million cells were transferred into a separate cluster tube for the unstained control. Leftover cells were pooled and put aside to generate single stain reference controls.

c. **Permeabilization**

Next, cells were permeabilized by washing the cells with 1x eBioscience permeabilization buffer followed by a 10 minute incubation in 1x eBioscience permeabilization buffer at room temperature.

d. **Antibody staining**

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Next, cells were stained with antibodies conjugated to fluorochromes. Each antibody clone recognizes a specific molecule on the cell surface. One antibody, gdTCR, is 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 in staining buffer. Cells are resuspended in 100 uL for storage at 4°C. DCS138 Cytek 25-Color Whole Blood Immunoprofiling Assay contains more details about this part of the procedure.

e. Data acquisition

Before acquisition, samples were resuspended in 300uL of staining buffer 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 a single-cell stream 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.

f. Data post-processing

Manual gating of FCS files was performed using CellEngine (CellCarta, Montreal, Canada). Fluorescence 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 with the exception of neutrophils and basophils, which are determined as % of total leukocytes. 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.

g. Data analysis

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

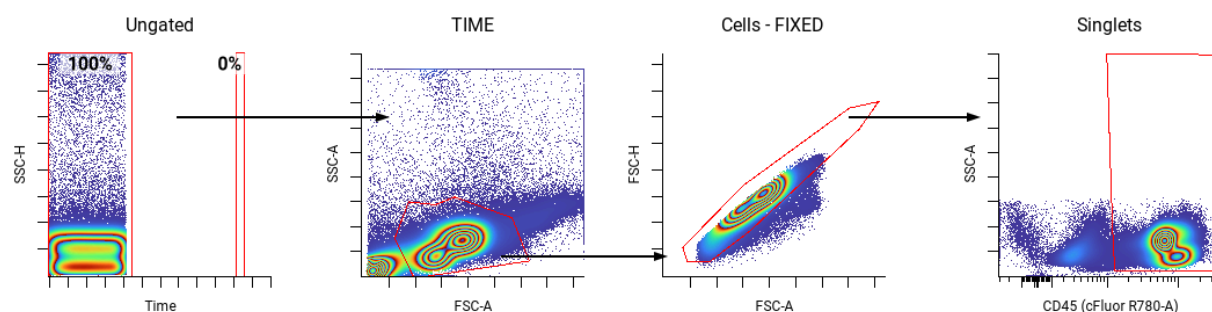
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For stability assessments, the percent change between each 1 month, 1 year accelerated, and 2 years accelerated time point when compared to Day 0 was calculated as follows: $\% \text{ change} = 100 * (\text{frequency at timepoint} - \text{frequency at } 0\text{h}) / \text{frequency at } 0\text{h}$. The data were visualized with R using the ggplot2 package to display the median percent change between Day 0 and 1 month, 1 year accelerated, and 2 years accelerated, 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: $\text{SEM} = \text{SD}(\text{percent change between Day 0 and timepoint}) / \sqrt{\text{number of samples}}$.

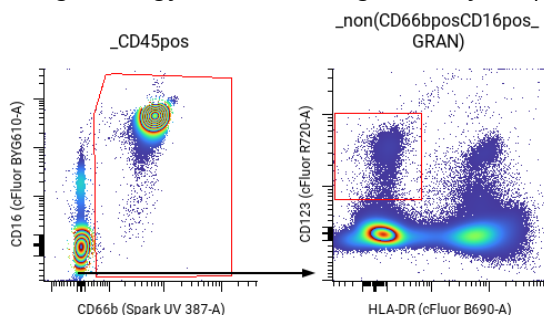
For the reference range experiment, we calculated the median, standard deviation, minimum, and maximum percent of frequency for each population.

Figure 1: Gating strategy used for Teiko Bio's 25-Marker WB Spectral Flow Pan-Immune Profiling Test

A. Gating strategy for total leukocytes (from total events)



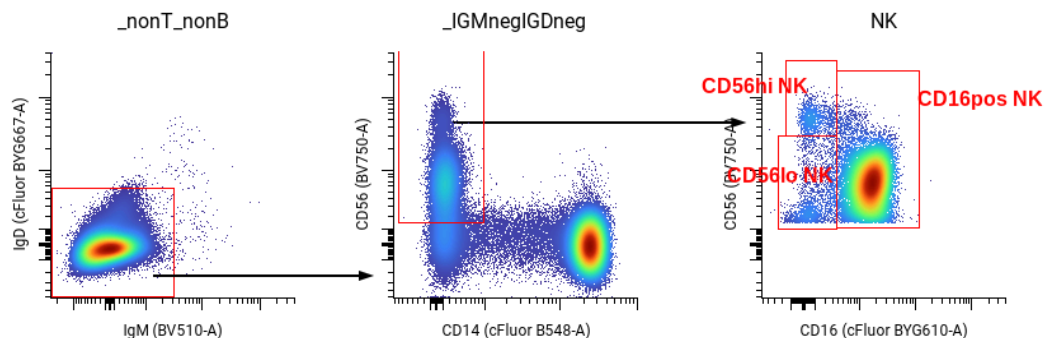
B. Gating strategy for total non-granulocytes (from total leukocytes)



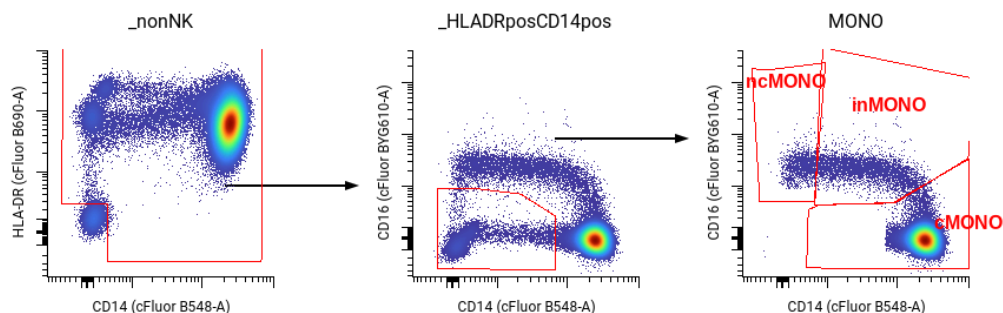
C. Gating strategy for B cells (from total non-granulocytes)

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

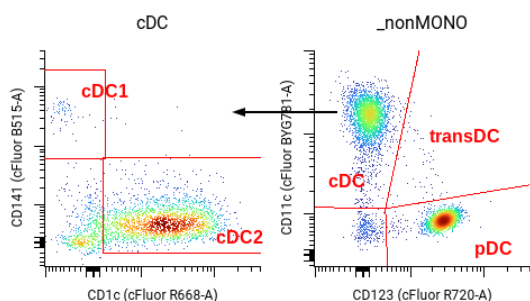
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F. Gating strategy for monocytes (from non-T, non-B and non-NK cells)



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. Limit of Blank (LOB)

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,

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fluorescence-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]:

- Nearby fluorophores (i.e. those close to each other on the emission spectrum) were distributed across FMM controls.
- Marker pairs used in bivariate plots were placed on different FMM controls (e.g. because CD27 and CD45RA are used in combination to gate on T cell populations, they are used in different controls).
- Activation markers were distributed across FMM controls (e.g. HLA-DR, CD25).
- 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 and CD8.
- 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 $\leq 1\%$ 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.

- Intra-run precision was assessed by taking whole blood from three (3) healthy donors (WB-011, WB-012, WB-013), 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 Cytex® Aurora machine during one run. Variance between replicates was calculated for precision analysis. **Acceptance criteria were $CV \leq 20\%$ for $\geq 95\%$ of all measurable (>100 cells median) phenotypic immune cell populations and $CV \leq 20\%$ for $\geq 90\%$ of functional marker subsets.**
- Inter-run precision was assessed by taking whole blood from three (3) healthy donors (WB-011, WB-012, WB-013) and splitting the sample into three technical replicates. Each replicate was processed (stained and run on Aurora) independently by three operators. Variance between runs was calculated for precision analysis. **Acceptance criteria were $CV \leq 25\%$ for $\geq 95\%$ of all measurable (>100 cells median) phenotypic immune cell populations and**

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CV ≤ 25% for ≥ 90% of functional marker subsets.

3. Stability

Post-fixation stability was evaluated to ensure test results are not affected by storage-related variables. Stability was assessed with whole blood specimens from three (3) healthy donors (WB-030, WB-031, WB-032). Whole blood was collected, processed with TokuKit, split into replicates for each time point, and stored at -80C. The sets of replicates were run on Day 0 and after 1 month in storage at -80C. 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 ≥ 90% of all measurable (>100 cell events) phenotypic immune populations and functional marker subsets from Day 0.**

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 processing whole blood specimens from six (6) healthy donors (WB-011, WB-012, WB-013, WB-030, WB-031, WB-032) with TokuKit and staining and fixing them by the same operator. Specimens were run on the Cytex® Aurora machine during one run. All steps were performed by the same operator. 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 whole blood specimen 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.

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Table 5. 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	IgM	IgM	IgM	-	IgM
20	CD3	CD3	CD3	CD3	CD3
21	CD28	-	CD28	CD28	CD28
22	CD38	CD38	-	CD38	CD38
23	CD56	CD56	CD56	-	CD56
24	CD66b	CD66b	-	CD66b	CD66b
25	CD279 (PD-1)	CD279 (PD-1)	CD279 (PD-1)	CD279 (PD-1)	-

Table 6: 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.

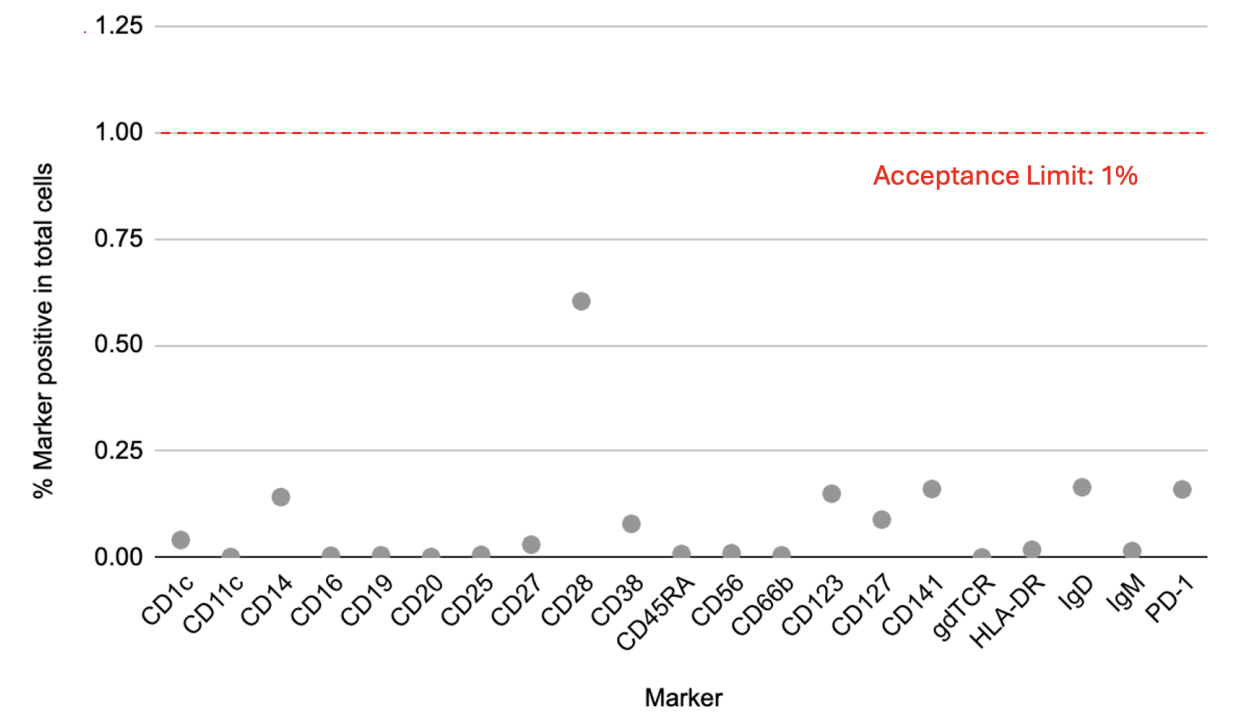
No.	Marker	% Marker Positive in Full Panel	% Marker Positive in Corresponding FMM
1	CD1c	2.14	0.041
2	CD11c	72.05	0.001

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3	CD14	38.99	0.142
4	CD16	62.3	0.004
5	CD19	3.58	0.005
6	CD20	3.53	0.001
7	CD25	1.05	0.006
8	CD27	21.84	0.030
9	CD28	77.94	0.603
10	CD3	N/A	N/A
11	CD38	15.49	0.079
12	CD4	N/A	N/A
13	CD45	N/A	N/A
14	CD45RA	36.08	0.008
15	CD56	4.19	0.010
16	CD66b	58.33	0.005
17	CD8	N/A	N/A
18	CD123	0.36	0.150
19	CD127	18.51	0.089
20	CD141	3.97	0.161
21	gdTCR	1.16	0.000
22	HLA-DR	14.52	0.018
23	IgD	3	0.165
24	IgM	5.67	0.015
25	PD-1	3.17	0.160

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



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2. Precision

a. Intra-Run

Intra-run precision was assessed by taking whole blood from three (3) healthy donors (WB-011, WB-012, WB-013), 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 Cytex® Aurora instrument during one run.

1. Phenotypic populations

Variance of phenotypic populations between replicates was calculated for precision analysis and can be seen in Tables 7-9 and Figure 3. 98.2% (109/111) of measurable (>100 cells) cell populations passed the acceptable threshold of $\leq 20\%$ variation between replicates. **Total median intra-run %CV for all measurable populations across all three donors was 3.38%. Total mean intra-run %CV for all measurable populations across all three donors was 5.19%.**

Table 7: Summary of Intra-Run Precision Assessment for Donor 1 (WB-011)

Cell Subset	# of Replicates	Median # of Cells measured	Average % of top Gate	%CV between replicates (%)
Basophils	3	7209	0.94	5.98
Neutrophils	3	441465	56.65	0.30
B cells	3	27197	8.40	2.36
Memory	3	2650	0.83	7.22
Memory Switched	3	2301	0.72	6.25
Memory Unswitched	3	397	0.12	15.08
Marginal Zone-like	3	5305	1.62	10.30
Naive	3	18317	5.58	2.70
Plasmablasts (PB)	2	106	0.03	13.61
T cells	3	198457	59.62	0.14
Gamma Delta ($\gamma\delta$) T cells	3	9541	2.95	2.58
Natural Killer T cells (NKT)	3	5219	1.66	6.39
CD4+ T cells	3	114814	34.57	0.19
Regulatory T cells (Treg)	3	5069	1.59	5.07

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nonTreg	3	109348	32.98	0.33
Naive	3	78557	23.71	2.81
Central Memory (TCM)	3	23352	7.35	8.35
Effector Memory (TEM)	3	3135	0.99	7.66
CD45RA+ Effector Memory (TEMRA)	3	3021	0.93	7.22
CD8+ T cells	3	64599	19.27	0.71
Naive	3	56008	16.76	0.68
Central Memory (TCM)	3	4091	1.26	3.38
Effector Memory (TEM)	3	329	0.10	8.65
CD45RA+ Effector Memory (TEMRA)	3	3822	1.15	2.89
CD4/CD8 Double-Negative T cells (DNT)	3	1802	0.59	17.05
CD4/CD8 Double-Positive T cells (DPT)	3	1921	0.58	1.87
Natural Killer (NK) cells	3	27831	8.39	1.18
CD16+	3	20682	6.41	4.59
CD16-	3	3644	1.17	11.48
CD56hi	3	2317	0.72	4.56
Monocytes	3	66722	20.09	1.20
Classical (cMono)	3	57625	17.30	0.51
Intermediate (inMono)	3	5774	1.78	5.99
Non-Classical (ncMono)	3	3196	0.99	5.76
Dendritic Cells	3	5996	1.83	1.26
Classical (cDC)	3	4500	1.35	1.44
Type 1 (cDC1)	0	67	0.02	*
Type 2 (cDC2)	3	1733	0.53	2.70
Plasmacytoid (pDC)	3	1444	0.46	8.09
Transitional (tDC)	0	25	0.01	*

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Donor 1 Median %CV				3.97
Donor 1 Mean %CV				4.96

**Populations with fewer than 100 cell events across all 3 replicates were excluded from precision analysis and CV calculations.*

Table 8: Summary of Intra-Run Precision Assessment for Donor 2 (WB-012)

Cell Subset	# of Replicates	Median # of Cells measured	Average % of top Gate	%CV between replicates (%)
Basophils	3	852	0.13	12.07
Neutrophils	3	485566	65.50	0.41
B cells	3	23425	9.10	6.80
Memory	3	2177	0.83	4.36
Memory Switched	3	1827	0.71	2.31
Memory Unswitched	3	331	0.13	16.46
Marginal Zone-like	3	7143	2.76	9.49
Naive	3	13144	5.14	5.93
Plasmablasts (PB)	0	32	0.01	*
T cells	3	139623	54.48	0.79
Gamma Delta ($\gamma\delta$) T cells	3	3595	1.42	2.45
Natural Killer T cells (NKT)	3	3576	1.40	1.27
CD4+ T cells	3	84300	33.12	1.22
Regulatory T cells (Treg)	3	5037	1.98	0.62
nonTreg	3	79206	31.14	1.30
Naive	3	30822	11.91	2.33
Central Memory (TCM)	3	44144	17.49	2.05
Effector Memory (TEM)	3	4234	1.73	4.46
CD45RA+ Effector Memory (TEMRA)	0	7	0.00	*
CD8+ T cells	3	43535	16.88	1.06
Naive	3	12905	4.99	1.56
Central Memory (TCM)	3	25213	9.81	0.73

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Effector Memory (TEM)	3	3333	1.29	1.13
CD45RA+ Effector Memory (TEMRA)	3	2057	0.79	2.04
CD4/CD8 Double-Negative T cells (DNT)	3	2373	0.98	5.33
CD4/CD8 Double-Positive T cells (DPT)	3	1721	0.68	2.04
Natural Killer (NK) cells	3	30507	11.82	1.96
CD16+	3	26080	10.00	6.54
CD16-	3	3326	1.37	25.60
CD56hi	3	927	0.38	6.40
Monocytes	3	54509	22.07	2.81
Classical (cMono)	3	50828	20.57	2.77
Intermediate (inMono)	3	2948	1.20	3.55
Non-Classical (ncMono)	3	826	0.33	3.54
Dendritic Cells	3	1695	0.72	8.95
Classical (cDC)	3	1420	0.60	10.99
Type 1 (cDC1)	0	17	0.01	*
Type 2 (cDC2)	3	551	0.22	5.59
Plasmacytoid (pDC)	3	253	0.10	8.38
Transitional (tDC)	0	34	0.01	*
Donor 2 Median %CV				2.79
Donor 2 Mean %CV				4.87

**Populations with fewer than 100 cell events across all 3 replicates were excluded from precision analysis and CV calculations.*

Table 9: Summary of Intra-Run Precision Assessment for Donor 3 (WB-013)

Cell Subset	# of Replicates	Median # of Cells measured	Average % of top Gate	%CV between replicates (%)
Basophils	3	6277	0.86	3.53
Neutrophils	3	410401	59.15	2.53

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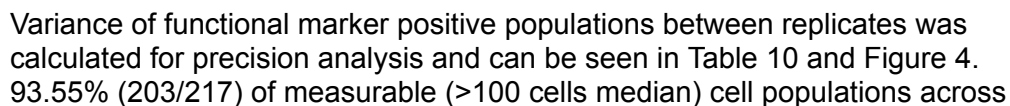
B cells	3	30223	10.72	2.15
Memory	3	2331	0.79	3.57
Memory Switched	3	2035	0.69	2.37
Memory Unswitched	3	257	0.09	13.24
Marginal Zone-like	3	2601	0.92	1.79
Naive	3	23620	8.44	2.76
Plasmablasts (PB)	3	269	0.09	17.05
T cells	3	138818	48.13	1.63
Gamma Delta ($\gamma\delta$) T cells	3	10543	3.63	1.59
Natural Killer T cells (NKT)	3	12911	4.38	4.12
CD4+ T cells	3	77640	26.72	2.13
Regulatory T cells (Treg)	3	7365	2.50	5.75
nonTreg	3	70098	24.22	1.81
Naive	3	10499	3.74	3.12
Central Memory (TCM)	3	54576	18.73	1.99
Effector Memory (TEM)	3	4986	1.74	1.44
CD45RA+ Effector Memory (TEMRA)	0	38	0.01	*
CD8+ T cells	3	32809	11.56	1.90
Naive	3	11338	4.05	2.67
Central Memory (TCM)	3	17895	6.22	2.46
Effector Memory (TEM)	3	1696	0.60	2.46
CD45RA+ Effector Memory (TEMRA)	3	1880	0.70	8.16
CD4/CD8 Double-Negative T cells (DNT)	3	3636	1.40	10.90
CD4/CD8 Double-Positive T cells (DPT)	3	1279	0.44	2.69
Natural Killer (NK) cells	3	42524	15.22	2.43
CD16+	3	37246	13.06	4.00

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CD16-	3	4211	1.75	36.07
CD56hi	3	883	0.31	5.54
Monocytes	3	65759	22.54	3.70
Classical (cMono)	3	58344	20.00	4.12
Intermediate (inMono)	3	3884	1.29	5.93
Non-Classical (ncMono)	3	3389	1.25	5.67
Dendritic Cells	3	4887	1.76	8.20
Classical (cDC)	3	2619	0.98	7.09
Type 1 (cDC1)	0	36	0.01	*
Type 2 (cDC2)	3	716	0.24	15.86
Plasmacytoid (pDC)	3	2200	0.77	9.76
Transitional (tDC)	0	6	0.00	*
Donor 3 Median %CV				3.53
Donor 3 Mean %CV				5.74

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

Intrarun



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three donors passed the acceptable threshold of $\leq 20\%$ variation between replicates. **Total median intra-run %CV for all measurable populations across all three donors was 5.50%. Total mean intra-run %CV for all measurable populations across all three donors was 7.90%.**

Table 10: Summary of Intra-Run Precision Assessment for Functional Marker Positive Populations across all three Donors

Parent Population > Functional Marker Subset	WB-011	WB-012	WB-013
B_CELL > CD25			37.93
B_CELL > PD1	15.85		11.78
B_MZ > CD25			36.39
B_NAIVE > CD25			44.07
B_NAIVE > PD1			5.35
CD16_NK > CD8	1.12	3.28	7.93
CD16_NK > HLADR	13.65	9.27	9.44
CD16_NK > PD1	29.33		45.72
CD16neg_NK > CD8	2.08	2.50	9.75
CD16neg_NK > HLADR	10.37	28.02	8.18
CD4_nonTREG > CD28	2.49	3.01	5.50
CD4_nonTREG > CD38	0.11	0.03	0.74
CD4_nonTREG > HLADR	6.96	6.81	5.16
CD4_nonTREG > PD1	7.15	7.15	4.31
CD4_T > CD28	2.44	2.80	5.86
CD4_T > CD38	0.14	0.03	0.80
CD4_T > HLADR	6.19	5.46	4.40
CD4_T > PD1	7.05	7.28	3.56
CD4_TCM > CD28	7.45	2.21	8.93
CD4_TCM > CD38	0.05	0.02	0.35
CD4_TCM > HLADR	7.27	9.06	6.67
CD4_TCM > PD1	7.29	5.85	4.58

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CD4_TEM > CD28	6.18	3.23	7.63
CD4_TEM > CD38	0.07	0.22	0.21
CD4_TEM > HLADR	8.29	5.43	1.96
CD4_TEM > PD1	7.97	7.55	3.96
CD4_TEMRA > CD28	4.45		
CD4_TEMRA > CD38	0.37		
CD4_TEMRA > HLADR	6.91		
CD4_TEMRA > PD1	6.12		
CD4_TNAIVE > CD28	2.01	2.48	2.93
CD4_TNAIVE > CD38	0.15	0.08	3.08
CD4_TNAIVE > HLADR	18.87		
CD4_TNAIVE > PD1	11.93	18.71	13.72
CD56hi_NK > CD8	4.71	9.14	12.14
CD56hi_NK > HLADR	2.55	10.89	
CD8_T > CD28	8.07	3.42	9.99
CD8_T > CD38	2.43	1.20	7.56
CD8_T > HLADR	3.80	10.37	4.37
CD8_T > PD1	5.18	3.47	3.61
CD8_TCM > CD28	10.59	3.55	7.71
CD8_TCM > CD38	1.26	1.08	2.40
CD8_TCM > HLADR	7.77	10.70	3.79
CD8_TCM > PD1	1.40	3.32	4.31
CD8_TEM > CD28		2.81	
CD8_TEM > CD38		5.31	2.21
CD8_TEM > HLADR		5.21	5.73
CD8_TEM > PD1	2.64	0.80	6.71
CD8_TEMRA > CD28	12.34	4.79	13.18
CD8_TEMRA > CD38	3.64		
CD8_TEMRA > HLADR	6.13		5.98

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CD8_TEMRA > PD1	7.99	11.42	
CD8_TNAIVE > CD28	7.80	3.35	11.77
CD8_TNAIVE > CD38	2.45	2.03	18.56
CD8_TNAIVE > HLADR	3.68	15.27	4.55
CD8_TNAIVE > PD1	6.77	7.34	8.29
cMONO > PD1	27.94		67.41
DNT > CD28	15.19	4.04	3.76
DNT > CD38	2.10	1.43	5.53
DNT > HLADR	6.15	6.79	7.04
DNT > PD1	12.69	3.05	5.55
DPT > CD28	6.32	3.64	17.66
DPT > CD38	0.99	0.46	4.26
DPT > PD1	2.64	2.00	2.59
GDT > CD28	7.10	21.49	67.06
GDT > HLADR	9.62		3.46
GDT > PD1	2.31	16.07	7.33
MONO > PD1	24.30		55.88
NEUTROPHIL > HLADR	5.51	12.79	13.33
NK > CD8	1.38	2.39	8.09
NK > HLADR	5.70	7.71	10.52
NK > PD1	25.17	17.67	37.47
NKT > CD28	4.00	2.51	9.61
NKT > CD38	0.88	3.76	6.55
NKT > HLADR			8.61
NKT > PD1	16.58	3.31	
T_CELL > CD28	3.14	2.55	10.78
T_CELL > CD38	0.63	0.40	2.48
T_CELL > HLADR	3.46	8.24	4.33
T_CELL > PD1	5.80	4.78	3.63

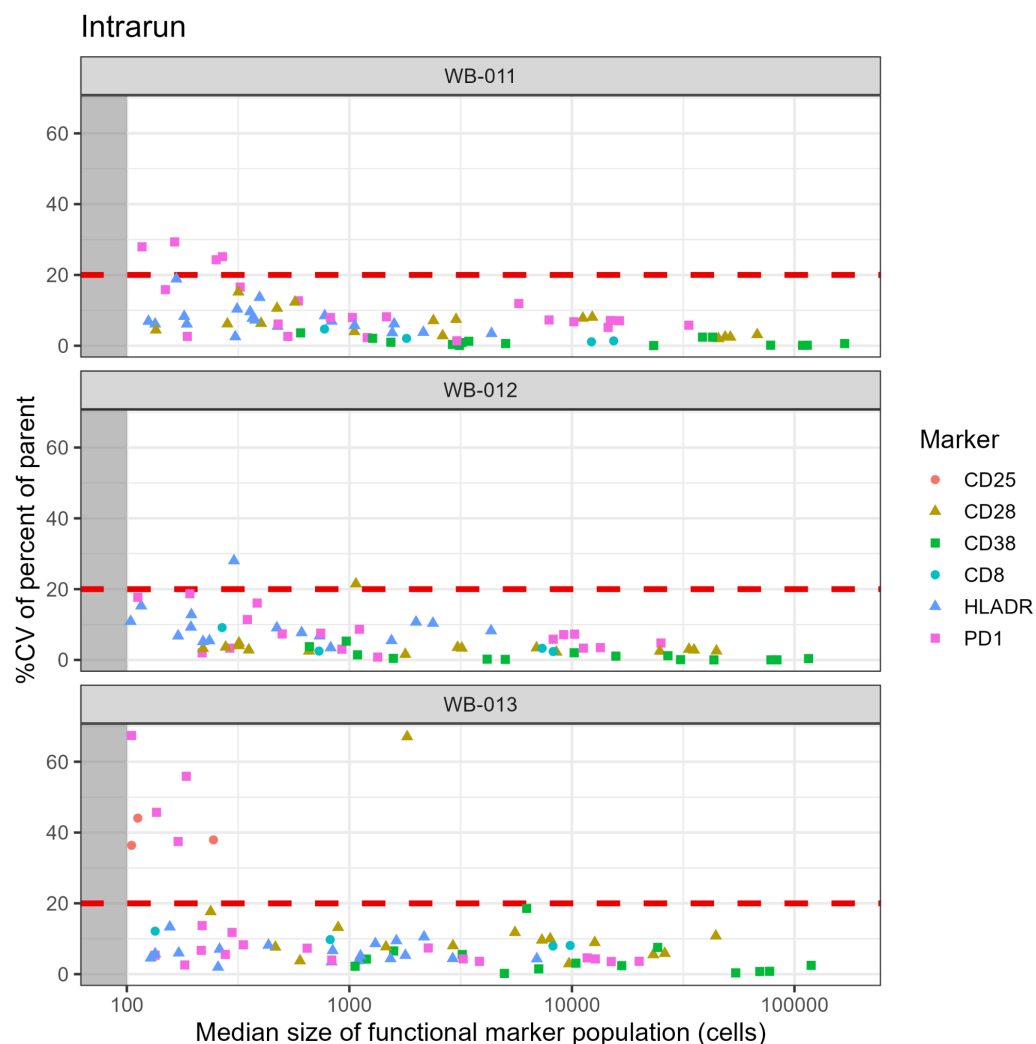
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TREG > CD28	2.84	1.58	8.02
TREG > CD38	0.63	0.16	1.49
TREG > HLADR	8.48	3.45	5.31
TREG > PD1	8.19	8.66	7.38
Median	5.96	3.55	6.61
Mean	6.73	5.74	11.07

**Only populations with more than 100 cell events across all 3 replicates were included within precision analysis and CV calculations.*

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Figure 4: Coefficient of Variation in Frequency (Percentage of Parent) and Median Number of Cells per Gated Functional Marker for Intra-Run Precision Assessment



b. Inter-Run

Inter-run precision was assessed by taking whole blood from three (3) healthy donors (WB-011, WB-012, WB-013) and splitting the sample into three technical replicates. Each replicate was processed (stained and run on Aurora) independently by three separate analysts.

1. Phenotypic populations

Variance of phenotypic populations between runs was calculated for precision analysis and can be seen in Tables 11-13 and Figure 5. 95.45% (105/110) of

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measurable (>100 cells) cell populations passed the acceptable threshold of $\leq 25\%$ variation between replicates. **Total median inter-run %CV for all measurable populations across all three donors was 4.56%. Total mean inter-run %CV for all measurable populations across all three donors was 7.92%.**

Table 11: Summary of Inter-Run Precision Assessment for Donor 1 (WB-011)

Cell Subset	# of Replicates	Median # of Cells measured	Average % of top Gate	%CV between replicates (%)
Basophils	3	11015	1.13	1.24
Neutrophils	3	562721	58.82	2.25
B cells	3	34555	8.63	8.43
Memory	3	3262	0.86	17.87
Memory Switched	3	2842	0.72	11.55
Memory Unswitched	3	420	0.14	53.55
Marginal Zone-like	3	6447	1.71	22.14
Naive	3	23486	5.72	5.38
Plasmablasts (PB)	1	86	0.02	*
T cells	3	234605	58.79	1.38
Gamma Delta ($\gamma\delta$) T cells	3	12248	2.97	3.89
Natural Killer T cells (NKT)	3	7617	1.80	7.92
CD4+ T cells	3	135310	33.96	1.34
Regulatory T cells (Treg)	3	6456	1.77	21.41
nonTreg	3	128854	32.19	2.58
Naive	3	96745	23.83	4.69
Central Memory (TCM)	3	26590	6.63	6.58
Effector Memory (TEM)	3	3507	0.83	6.50
CD45RA+ Effector Memory (TEMRA)	3	3877	0.92	10.73
CD8+ T cells	3	75072	19.01	0.93
Naive	3	65402	16.58	0.41

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Central Memory (TCM)	3	5173	1.22	9.71
Effector Memory (TEM)	3	371	0.08	28.31
CD45RA+ Effector Memory (TEMRA)	3	4434	1.14	2.20
CD4/CD8 Double-Negative T cells (DNT)	3	1930	0.47	3.94
CD4/CD8 Double-Positive T cells (DPT)	3	2428	0.59	4.28
Natural Killer (NK) cells	3	34983	8.70	1.61
CD16+	3	31066	7.67	2.92
CD16-	3	1077	0.27	10.53
CD56hi	3	2742	0.74	9.70
Monocytes	3	79946	20.67	3.63
Classical (cMono)	3	67859	17.58	3.43
Intermediate (inMono)	3	8299	2.08	7.06
Non-Classical (ncMono)	3	3869	1.00	4.44
Dendritic Cells	3	5579	1.39	4.02
Classical (cDC)	3	3030	0.78	10.27
Type 1 (cDC1)	1	94	0.03	*
Type 2 (cDC2)	3	2266	0.58	14.47
Plasmacytoid (pDC)	3	2489	0.60	8.60
Transitional (tDC)	0	47	0.01	*
Donor 1 Median				5.38
Donor 1 Mean				8.65

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

Table 12: Summary of Inter-Run Precision Assessment for Donor 2 (WB-012)

Cell Subset	# of Replicates	Median # of Cells measured	Average % of top Gate	%CV between replicates (%)
Basophils	3	2032	0.20	5.19

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Neutrophils	3	652750	68.38	1.46
B cells	3	31387	9.58	11.38
Memory	3	2964	0.86	16.87
Memory Switched	3	2520	0.74	13.55
Memory Unswitched	3	444	0.13	39.26
Marginal Zone-like	3	10732	3.47	23.31
Naive	3	16762	4.99	8.03
Plasmablasts (PB)	1	38	0.02	*
T cells	3	166336	53.59	1.65
Gamma Delta ($\gamma\delta$) T cells	3	4217	1.37	3.67
Natural Killer T cells (NKT)	3	4838	1.52	7.83
CD4+ T cells	3	101413	32.63	1.67
Regulatory T cells (Treg)	3	6419	2.10	9.44
nonTreg	3	94994	30.53	2.36
Naive	3	38201	12.41	0.28
Central Memory (TCM)	3	51849	16.50	4.14
Effector Memory (TEM)	3	4999	1.62	4.12
CD45RA+ Effector Memory (TEMRA)	0	27	0.01	*
CD8+ T cells	3	51205	16.53	0.99
Naive	3	15000	4.98	2.98
Central Memory (TCM)	3	29885	9.51	3.35
Effector Memory (TEM)	3	4006	1.26	5.14
CD45RA+ Effector Memory (TEMRA)	3	2314	0.77	10.16
CD4/CD8 Double-Negative T cells (DNT)	3	2757	0.88	2.50
CD4/CD8 Double-Positive T cells (DPT)	3	2109	0.67	5.85
Natural Killer (NK) cells	3	37147	11.98	1.40

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CD16+	3	34725	11.01	2.70
CD16-	3	1776	0.57	54.59
CD56hi	3	1132	0.36	6.23
Monocytes	3	66894	22.63	5.31
Classical (cMono)	3	62300	21.08	4.98
Intermediate (inMono)	3	3647	1.25	10.95
Non-Classical (ncMono)	3	878	0.28	11.95
Dendritic Cells	3	1626	0.53	1.72
Classical (cDC)	3	1228	0.40	3.69
Type 1 (cDC1)	0	25	0.01	*
Type 2 (cDC2)	3	646	0.23	19.92
Plasmacytoid (pDC)	3	425	0.13	17.75
Transitional (tDC)	0	6	0.00	*
Donor 2 Median				5.17
Donor 2 Mean				9.07

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

Table 13: Summary of Inter-Run Precision Assessment for Donor 3 (WB-013)

Cell Subset	# of Replicates	Median # of Cells measured	Average % of top Gate	%CV between replicates (%)
Basophils	3	9384	0.98	3.14
Neutrophils	3	597621	63.97	1.91
B cells	3	36701	10.84	0.83
Memory	3	2758	0.82	0.81
Memory Switched	3	2456	0.72	2.75
Memory Unswitched	3	332	0.10	20.07
Marginal Zone-like	3	2932	0.91	10.30
Naive	3	30032	8.79	2.41
Plasmablasts (PB)	3	193	0.07	43.25

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T cells	3	157889	46.60	1.34
Gamma Delta ($\gamma\delta$) T cells	3	12098	3.54	2.60
Natural Killer T cells (NKT)	3	16558	4.73	5.84
CD4+ T cells	3	86855	25.67	0.82
Regulatory T cells (Treg)	3	7998	2.40	7.78
nonTreg	3	78857	23.28	1.54
Naive	3	13688	4.07	2.30
Central Memory (TCM)	3	59658	17.58	1.98
Effector Memory (TEM)	3	5784	1.60	7.55
CD45RA+ Effector Memory (TEMRA)	1	74	0.03	*
CD8+ T cells	3	37642	11.15	0.61
Naive	3	13691	4.05	0.85
Central Memory (TCM)	3	20027	5.92	0.79
Effector Memory (TEM)	3	2014	0.56	12.42
CD45RA+ Effector Memory (TEMRA)	3	1975	0.62	15.60
CD4/CD8 Double-Negative T cells (DNT)	3	3728	1.10	1.20
CD4/CD8 Double-Positive T cells (DPT)	3	1426	0.40	6.83
Natural Killer (NK) cells	3	52931	15.57	1.92
CD16+	3	48865	14.34	2.49
CD16-	3	2415	0.76	11.54
CD56hi	3	1515	0.44	3.17
Monocytes	3	76772	23.59	5.42
Classical (cMono)	3	66619	20.43	5.36
Intermediate (inMono)	3	6782	2.10	10.26
Non-Classical (ncMono)	3	3516	1.03	3.73
Dendritic Cells	3	5467	1.59	2.90

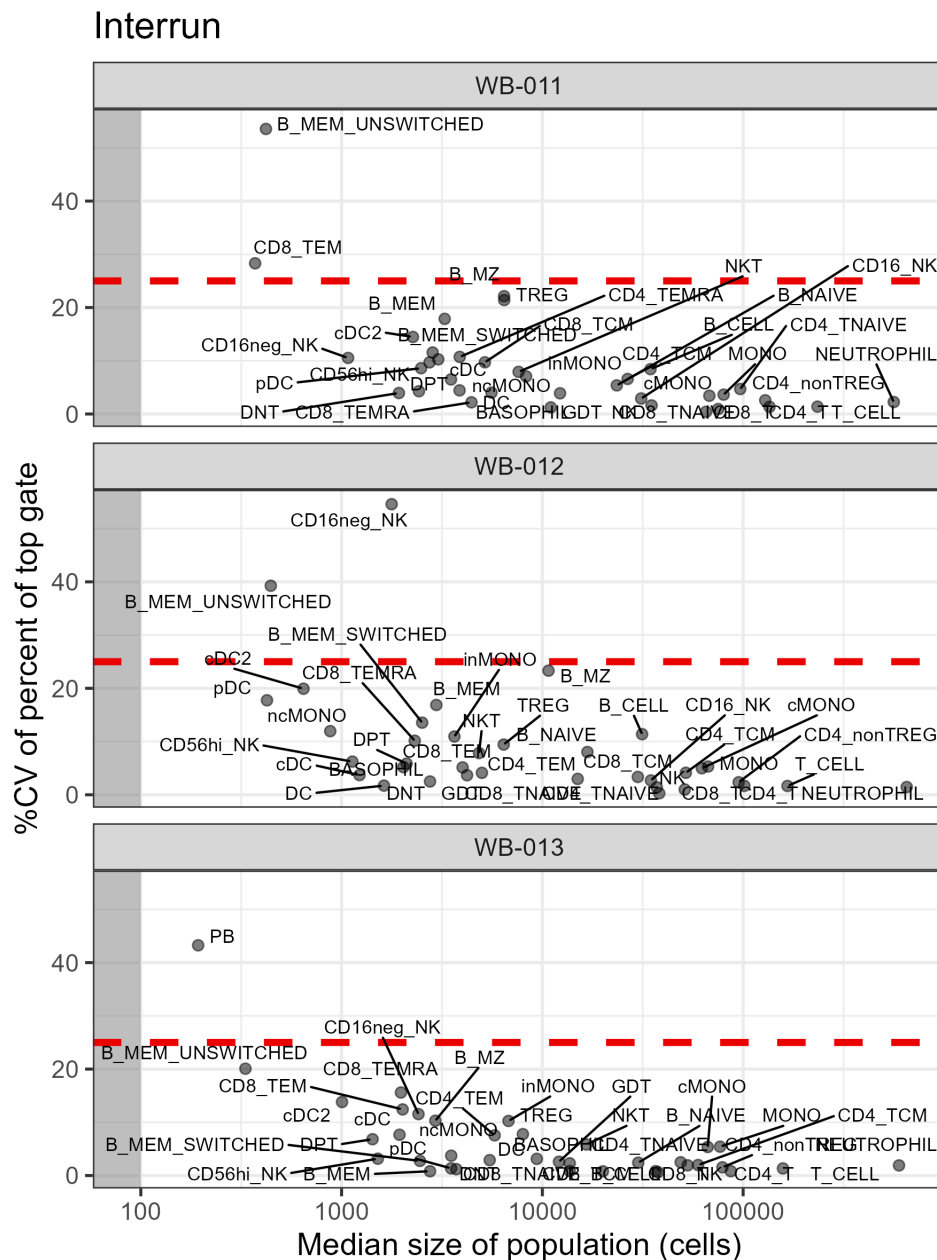
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Classical (cDC)	3	1943	0.53	7.66
Type 1 (cDC1)	1	60	0.02	*
Type 2 (cDC2)	3	1005	0.30	13.81
Plasmacytoid (pDC)	3	3511	1.05	1.35
Transitional (tDC)	0	10	0.00	*
Donor 3 Median				2.90
Donor 3 Mean				6.09

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

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Figure 5: Coefficient of Variation in Frequency (Percentage of Non-Granulocytes) and Median Number of Cells per Immune Population for Inter-Run Precision Assessment



2. Functional Markers

Variance of functional marker positive populations between replicates was calculated for precision analysis and can be seen in Table 14 and Figure 6. 90.87% (199/219) of measurable (>100 cells median) cell populations passed the

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acceptable threshold of $\leq 25\%$ variation between replicates. **Total median inter-run %CV for all measurable populations across all three donors was 7.28%. Total mean inter-run %CV for all measurable populations across all three donors was 12.32%.**

Table 14: Summary of Inter-Run Precision Assessment for Functional Marker Positive Populations across all three Donors

Parent Population > Functional Marker Subset	WB-011	WB-012	WB-013
B_CELL > CD25		82.47	54.55
B_CELL > PD1	32.27		13.55
B_NAIVE > PD1			9.09
CD16_NK > CD8	1.22	4.51	2.65
CD16_NK > HLADR	20.30	27.28	36.72
CD16_NK > PD1		30.20	15.66
CD16neg_NK > CD8	13.80	19.34	7.67
CD16neg_NK > HLADR	8.18	39.97	20.29
CD4_nonTREG > CD28	1.89	5.09	5.97
CD4_nonTREG > CD38	0.04	0.10	0.26
CD4_nonTREG > HLADR	6.75	4.15	4.96
CD4_nonTREG > PD1	7.46	17.25	15.55
CD4_T > CD28	2.09	5.15	5.37
CD4_T > CD38	0.07	0.12	0.35
CD4_T > HLADR	6.49	4.13	1.99
CD4_T > PD1	5.85	17.51	15.81
CD4_TCM > CD28	5.22	15.16	10.19
CD4_TCM > CD38	0.08	0.18	0.08
CD4_TCM > HLADR	12.75	3.20	4.23
CD4_TCM > PD1	9.25	15.55	15.12
CD4_TEM > CD28	4.53	13.59	15.12
CD4_TEM > CD38	0.12	0.18	0.12

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CD4_TEM > HLADR	5.06	5.21	4.55
CD4_TEM > PD1	8.32	15.20	10.77
CD4_TEMRA > CD28	3.04		
CD4_TEMRA > CD38	0.51		
CD4_TEMRA > HLADR	10.28		
CD4_TEMRA > PD1	3.22		
CD4_TNAIVE > CD28	2.95	3.83	3.08
CD4_TNAIVE > CD38	0.04	0.03	0.99
CD4_TNAIVE > HLADR	13.18		
CD4_TNAIVE > PD1	15.10	28.79	22.61
CD56hi_NK > CD8	6.82	8.46	10.46
CD56hi_NK > HLADR	11.26	23.36	11.34
CD8_T > CD28	9.80	8.87	6.33
CD8_T > CD38	0.60	2.15	3.60
CD8_T > HLADR	12.46	5.85	4.68
CD8_T > PD1	1.43	7.31	7.41
CD8_TCM > CD28	8.70	5.24	3.39
CD8_TCM > CD38	2.62	1.17	0.36
CD8_TCM > HLADR	11.71	7.28	5.84
CD8_TCM > PD1	0.87	6.06	7.81
CD8_TEM > CD28		11.99	7.08
CD8_TEM > CD38	29.13	2.70	5.94
CD8_TEM > HLADR		8.20	7.14
CD8_TEM > PD1	12.74	4.23	10.06
CD8_TEMRA > CD28	11.14	16.00	9.82
CD8_TEMRA > CD38	4.30		
CD8_TEMRA > HLADR	7.40		23.99
CD8_TEMRA > PD1	10.33	4.82	
CD8_TNAIVE > CD28	9.56	13.01	7.90

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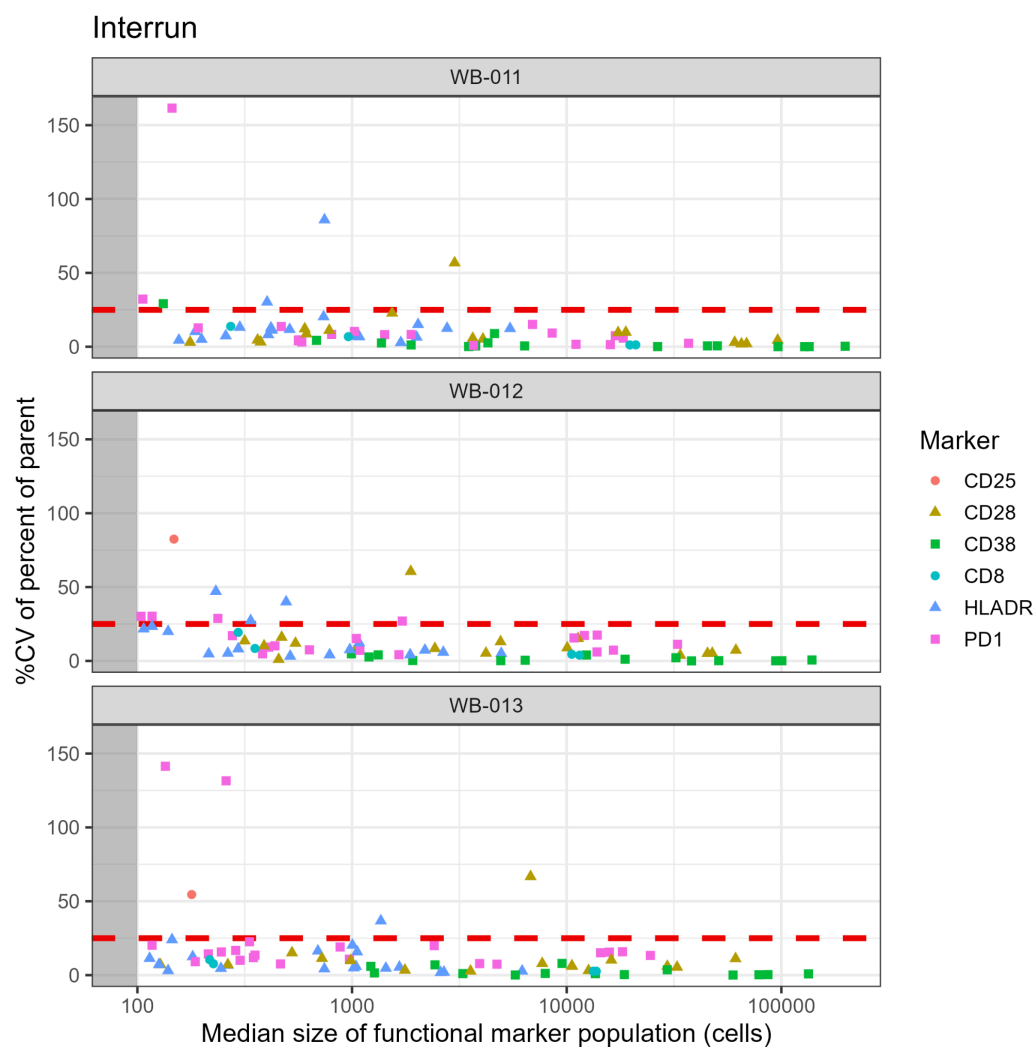
CD8_TNAIVE > CD38	0.56	4.00	7.92
CD8_TNAIVE > HLADR	15.07	19.95	3.04
CD8_TNAIVE > PD1	1.67	7.49	7.56
cMONO > PD1			141.39
DNT > CD28	3.22	1.04	11.36
DNT > CD38	2.54	4.09	1.02
DNT > HLADR	4.44	4.75	6.70
DNT > PD1	4.01	7.16	11.85
DPT > CD28	12.15	10.15	7.03
DPT > CD38	1.23	0.28	1.47
DPT > PD1	4.67	17.12	14.38
GDT > CD28	56.79	60.57	66.70
GDT > HLADR	30.25		16.31
GDT > PD1	8.18	9.33	19.03
MONO > PD1	161.53		131.56
NEUTROPHIL > HLADR	85.93	47.10	12.46
NK > CD8	1.27	3.90	2.66
NK > HLADR	2.82	7.63	1.79
NK > PD1		30.25	16.71
NKT > CD28	22.79	9.14	5.97
NKT > CD38	8.91	4.82	6.93
NKT > HLADR		21.67	15.77
NKT > PD1	13.68	10.22	20.22
T_CELL > CD28	4.46	7.26	11.16
T_CELL > CD38	0.29	0.65	0.83
T_CELL > HLADR	12.33	5.04	2.74
T_CELL > PD1	2.32	11.26	13.31
TREG > CD28	5.66	8.43	2.72
TREG > CD38	0.53	0.47	1.14

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TREG > HLADR	7.15	12.23	5.40
TREG > PD1	8.25	26.99	19.97
Median	6.62	7.40	7.56
Mean	11.27	12.11	13.57

**Only populations with more than 100 cell events across all 3 replicates were included within precision analysis and CV calculations.*

Figure 6: Coefficient of Variation in Frequency (Percentage of Parent) and Median Number of Cells per Gated Functional Marker for Inter-Run Precision Assessment



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3. Stability

Post-fixation stability was evaluated to ensure test results are not affected by storage-related variables. Stability was assessed by taking whole blood from three (3) healthy donors (WB-030, WB-031, WB-032). Samples were split into multiple replicates and were stored at -80°C. One set of replicates was run after the blood was thoroughly frozen (t=0). The other sets of replicates were run after one month of storage at -80°C (t=1M). All steps were performed by the same operator. Percentage of change in immune population frequencies was calculated in comparison to the t=0 time point for stability analysis and can be seen in Table 15 and Figure 7. Percentage of change in functional marker frequencies was calculated in comparison to the t=0 time point for stability analysis and can be seen in Table 16 and Figure 8-9.

1. Phenotypic populations:

The percentage of measurable immune populations across three donors with <25% change was 90.4% (103/114) for 1-month stability. **The median percentage of change of all measurable immune populations (>100 cell events) across three donors are 6.51%. The mean percentage of change of all measurable immune populations (>100 cell events) across three donors are 12.43%.**

Table 15: Summary of Stability Assessment for 1-Month Storage Across 3 Donors

Cell Subset	# of Subjects Measured	Median # of Cells (t=0)	Median # of Cells (t=1M)	Median % of Top Gate (t=0)	Median % of Top Gate t=1M)	Mean % Change
Basophils	3	4402	5730	0.52	0.59	11.91%
Neutrophils	3	598353	630259	62.10	65.07	2.25%
B cells	3	32549	39064	9.39	9.63	2.46%
Memory	3	4357	5548	1.21	1.31	6.37%
Memory Switched	3	3554	4029	0.98	0.95	6.64%
Memory Unswitched	3	914	1149	0.31	0.35	26.37%
Marginal Zone-like	3	6378	6512	2.08	1.83	11.80%
Naive	3	20196	25004	5.90	6.43	5.32%
Plasmablasts (PB)	2	302	23	0.08	0.01	86.64%
T cells	3	191422	172405	57.12	57.47	2.22%
Gamma Delta (γδ) T	3	6528	6930	2.45	2.57	3.34%

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cells						
Natural Killer T cells (NKT)	3	3277	3613	0.98	0.89	8.53%
CD4+ T cells	3	123774	124790	34.18	34.19	2.15%
Regulatory T cells (Treg)	3	9772	10027	2.70	2.36	7.97%
nonTreg	3	114002	112395	32.38	32.56	2.05%
Naive	3	63080	61725	17.48	17.87	1.05%
Central Memory (TCM)	3	52296	48241	14.44	13.62	5.03%
Effector Memory (TEM)	3	4706	4550	1.30	1.36	2.50%
CD45RA+ Effector Memory (TEMRA)	1	65	115	0.02	0.03	*
CD8+ T cells	3	48607	49534	18.26	18.37	2.17%
Naive	3	35032	41157	9.67	9.70	1.76%
Central Memory (TCM)	3	10329	9017	2.86	2.70	16.56%
Effector Memory (TEM)	3	1710	1522	0.47	0.46	20.88%
CD45RA+ Effector Memory (TEMRA)	3	2438	2930	0.92	1.09	11.73%
CD4/CD8 Double-Negative T cells (DNT)	3	1152	1160	0.43	0.43	2.85%
CD4/CD8 Double-Positive T cells (DPT)	3	1564	1522	0.59	0.55	16.60%
Natural Killer (NK) cells	3	20675	21705	5.71	5.39	5.45%
CD16+	3	17166	18483	4.74	4.36	3.14%
CD16-	3	2106	1972	0.67	0.52	16.81%

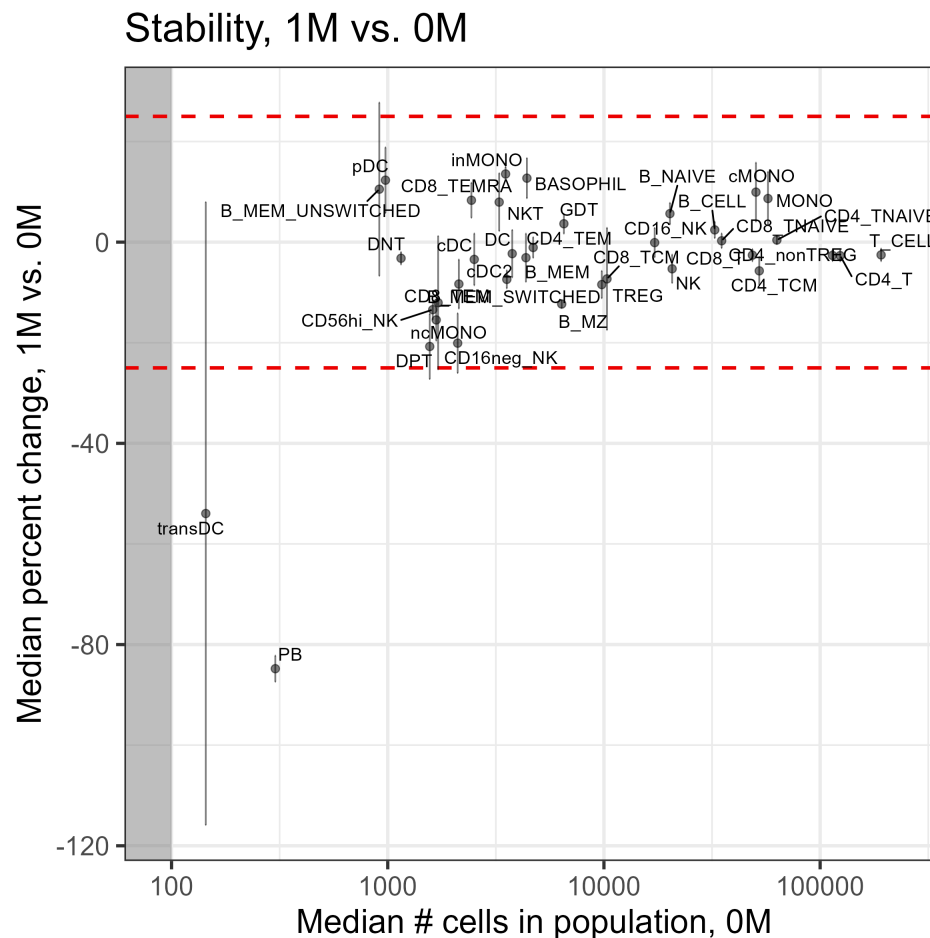
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CD56hi	3	1615	1416	0.52	0.46	14.20%
Monocytes	3	57372	66976	15.90	17.28	8.93%
Classical (cMono)	3	50477	59360	13.99	15.92	9.92%
Intermediate (inMono)	3	3509	4302	0.97	1.10	12.20%
Non-Classical (ncMono)	3	1676	1526	0.63	0.47	16.95%
Dendritic Cells	3	3765	3208	1.19	1.19	6.19%
Classical (cDC)	3	2513	2263	0.72	0.69	7.13%
Type 1 (cDC1)	0	59	77	0.02	0.02	*
Type 2 (cDC2)	3	2133	1796	0.60	0.54	11.15%
Plasmacytoid (pDC)	3	976	1013	0.34	0.33	11.46%
Transitional (tDC)	2	144	60	0.04	0.02	81.56%
Median						6.51%
Mean						12.43%

**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.*

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Figure 7: Median Percentage of Change in Frequency (Percentage of top level gate) and Median Number of Cells per Immune Population for Stability Assessment at one month (t=1M) compared to 0 month (t=0).



2. Functional Markers

The percentage of measurable immune populations across three donors with <25% change was 80.37% (103/114) for 1-month stability. **The median percentage of change of all measurable immune populations (>100 cell events) across three donors are 8.36%. The mean percentage of change of all measurable immune populations (>100 cell events) across three donors are 14.93%.**

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Table 16: Summary of Stability Assessment for 1-Month Storage Across 3 Donors

Cell Subset	# of Subjects Measured	Median # of Cells (t=0)	Median # of Cells (t=1M)	Median % of Top Gate (t=0)	Median % of Top Gate t=1M)	Mean % Change
BASOPHIL > HLADR	2	299	398	6.13	6.95	32.29%
CD16_NK > CD8	3	6839	7303	44.41	49.58	8.19%
CD16_NK > HLADR	3	939	1688	5.47	9.13	74.34%
CD16neg_NK > CD8	3	475	245	20.15	17.63	15.63%
CD16neg_NK > HLADR	3	1013	1026	49.70	52.43	17.95%
CD4_nonTREG > CD28	3	112661	112243	99.89	99.86	0.04%
CD4_nonTREG > CD38	3	59889	70006	52.53	56.40	4.74%
CD4_nonTREG > HLADR	3	4149	4045	3.32	3.60	10.74%
CD4_nonTREG > PD1	3	5106	5303	4.59	4.72	9.91%
CD4_T > CD28	3	122395	124636	99.89	99.88	0.04%
CD4_T > CD38	3	63499	73688	51.30	55.10	4.61%
CD4_T > HLADR	3	6577	7200	5.31	5.15	4.10%
CD4_T > PD1	3	5813	5835	4.66	4.68	10.44%
CD4_TCM > CD28	3	52140	48130	99.82	99.77	0.03%
CD4_TCM > CD38	3	13934	14424	26.64	27.52	5.85%
CD4_TCM > HLADR	3	1495	1781	2.86	3.10	11.56%
CD4_TCM > PD1	3	4649	4751	14.46	13.57	11.66%
CD4_TEM > CD28	3	4690	4522	99.66	99.38	0.46%
CD4_TEM > CD38	2	167	217	3.55	4.77	33.64%
CD4_TEM > HLADR	3	1374	1399	29.20	30.75	11.89%
CD4_TEM > PD1	3	394	448	8.37	9.85	10.39%
CD4_TNAIVE > CD28	3	63066	61714	99.95	99.98	0.02%
CD4_TNAIVE > CD38	3	44981	55362	84.91	90.40	4.21%
CD4_TNAIVE > HLADR	2	131	118	0.21	0.20	12.93%

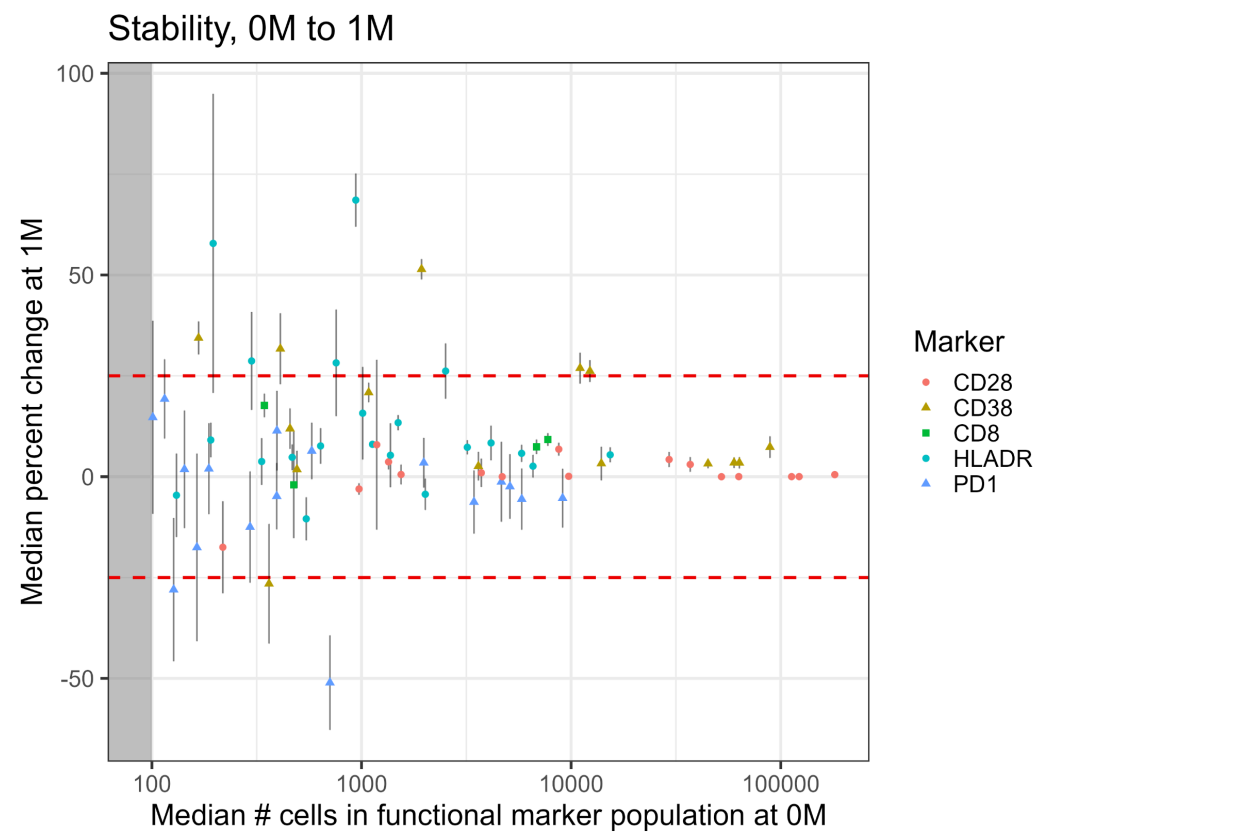
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CD4_TNAIVE > PD1	2	101	135	0.19	0.22	31.37%
CD56hi_NK > CD8	3	344	344	18.45	22.50	17.14%
CD56hi_NK > HLADR	3	757	851	46.87	60.10	37.43%
CD8_T > CD28	3	36999	38734	76.12	78.20	4.69%
CD8_T > CD38	3	12293	13963	24.20	31.90	26.85%
CD8_T > HLADR	3	5806	6259	12.55	13.83	6.24%
CD8_T > PD1	3	3440	3274	7.08	6.70	9.96%
CD8_TCM > CD28	3	8731	7856	84.53	87.12	6.11%
CD8_TCM > CD38	3	1082	1092	10.48	12.11	20.11%
CD8_TCM > HLADR	3	3197	3116	22.22	23.83	8.24%
CD8_TCM > PD1	3	1982	2072	27.18	27.09	7.86%
CD8_TEM > CD28	2	1182	1028	37.22	61.54	25.18%
CD8_TEM > HLADR	2	467	445	27.31	29.24	5.09%
CD8_TEM > PD1	2	294	287	17.19	11.19	20.05%
CD8_TEMRA > CD28	3	218	228	9.08	4.99	23.06%
CD8_TEMRA > CD38	3	410	649	16.82	22.15	27.97%
CD8_TEMRA > HLADR	3	638	721	32.53	36.73	7.56%
CD8_TEMRA > PD1	2	143	175	3.71	3.46	17.23%
CD8_TNAIVE > CD28	3	29340	36092	83.75	91.57	5.67%
CD8_TNAIVE > CD38	3	11031	12649	33.77	45.40	27.53%
CD8_TNAIVE > HLADR	3	1128	1334	3.86	4.17	8.69%
CD8_TNAIVE > PD1	3	395	469	1.13	1.14	15.64%
DNT > CD28	3	973	936	92.36	89.55	2.59%
DNT > CD38	3	492	448	18.31	20.55	5.82%
DNT > HLADR	3	334	288	12.12	13.62	7.86%
DNT > PD1	2	187	181	9.25	12.25	12.77%
DPT > CD28	3	1346	1358	86.06	89.22	5.37%
DPT > CD38	3	456	353	25.14	31.71	16.20%
DPT > PD1	3	115	130	7.98	8.54	18.94%

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GDT > CD28	3	3729	3669	57.12	52.94	4.25%
GDT > CD38	3	1934	2723	16.31	24.70	51.37%
GDT > HLADR	3	545	531	8.64	7.74	9.50%
GDT > PD1	3	579	590	4.41	4.56	9.69%
inMONO > PD1	3	127	140	3.62	2.51	29.82%
MONO > PD1	3	164	211	0.29	0.32	32.60%
NEUTROPHIL > HLADR	3	196	314	0.03	0.05	72.78%
NK > CD8	3	7742	7937	41.76	46.89	9.47%
NK > HLADR	3	2519	3175	12.18	14.63	29.74%
NKT > CD28	3	1544	1677	52.57	49.30	2.79%
NKT > CD38	3	362	366	13.19	9.69	24.48%
NKT > HLADR	3	191	231	7.62	9.24	12.70%
T_CELL > CD28	3	181009	163859	89.68	90.07	1.10%
T_CELL > CD38	3	88844	91590	36.09	41.49	9.65%
T_CELL > HLADR	3	15358	14615	8.02	8.48	3.70%
T_CELL > PD1	3	9105	9284	5.45	5.38	9.81%
TREG > CD28	3	9734	10026	99.87	99.98	0.18%
TREG > CD38	3	3610	3682	36.94	38.25	4.54%
TREG > HLADR	3	2015	1762	20.62	17.57	7.12%
TREG > PD1	3	707	470	6.01	4.29	39.42%
Median						8.36%
Mean						14.93%

Figure 8: Median Percentage of Change in Frequency (Percentage of parent) and Median Number of Cells per Functional State Marker for Stability Assessment at 1 month (t=1M) compared to 0 month (t=0).



Correlation of population frequencies, 1M vs. 0M

$R = 0.99$, $p < 0.000000000000000022$

$y = 1.1 + x$

Marker

- CD28
- CD38
- CD8
- HLA-DR
- PD1

Top level gate

- ▲ GRAN
- nonGRAN

Three scatter plots showing the relationship between the percent of parent population (1M) and the percent of parent population (0M) for three different populations: WB-030, WB-031, and WB-032. Each plot includes a solid black regression line, a dashed red line representing the identity line ($y=x$), and a dotted red line representing a steeper regression line. The y-axis is labeled 'Percent of parent population, 1M' and the x-axis is labeled 'Percent of parent population, 0M'. The regression equations are: WB-030: $R = 0.99, p < 0.00000000000000022, y = 0.74 + x$; WB-031: $R = 0.99, p < 0.00000000000000022, y = 1.7 + x$; WB-032: $R = 0.99, p < 0.00000000000000022, y = 0.84 + x$.

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4. Reference Range

Reference intervals were established using six healthy donors to determine baseline frequencies of all major immune cell lineages and subsets. All steps were performed by the same operator. Donors were of unknown sex and age. Average, standard deviation and range was calculated for each major immune population and are listed in Table 17.

Table 17: Reference Range Values for PBMCs from Healthy Subjects

Cell Subset	# of Subjects Measured	Average % Top Gate	Standard Deviation of % Top Gate	Range of % Top Gate
Basophils	6	0.58	0.29	0.13 - 0.94
Neutrophils	6	61.54	6.62	53.7 - 72.12
B cells	6	10.63	3.30	8.4 - 17.19
Memory	6	1.34	0.93	0.79 - 3.2
Memory Switched	6	1.14	0.86	0.69 - 2.88
Memory Unswitched	6	0.20	0.11	0.09 - 0.34
Marginal Zone-like	6	1.93	0.65	0.92 - 2.76
Naive	6	6.95	2.33	5.14 - 11.04
Plasmablasts (PB)	6	0.06	0.04	0.01 - 0.12
T cells	6	56.73	6.75	48.13 - 67.99
Gamma Delta ($\gamma\delta$) T cells	6	3.48	2.93	1.26 - 9.17
Natural Killer T cells (NKT)	6	1.72	1.34	0.69 - 4.38
CD4+ T cells	6	33.54	3.81	26.72 - 38.46
Regulatory T cells (Treg)	6	2.40	0.82	1.59 - 3.84
nonTreg	6	31.14	3.61	24.22 - 34.63
Naive	6	15.89	7.65	3.74 - 23.86
Central Memory (TCM)	6	13.47	5.10	6.97 - 18.73
Effector Memory (TEM)	6	1.49	0.58	0.81 - 2.39
CD45RA+ Effector Memory (TEMRA)	6	0.28	0.43	0 - 0.93
CD8+ T cells	6	16.68	4.18	11.56 - 22.13
Naive	6	9.93	5.40	4.05 - 16.76

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Central Memory (TCM)	6	4.87	3.62	1.23 - 9.81
Effector Memory (TEM)	6	0.89	1.02	0.1 - 2.76
CD45RA+ Effector Memory (TEMRA)	6	0.99	0.47	0.54 - 1.85
CD4/CD8 Double-Negative T cells (DNT)	6	0.75	0.41	0.28 - 1.4
CD4/CD8 Double-Positive T cells (DPT)	6	0.56	0.13	0.38 - 0.69
Natural Killer (NK) cells	6	9.71	3.78	5.69 - 15.22
CD16+	6	8.12	3.48	4.35 - 13.06
CD16-	6	1.04	0.47	0.58 - 1.75
CD56hi	6	0.48	0.16	0.31 - 0.72
Monocytes	6	20.22	4.10	15.04 - 25.66
Classical (cMono)	6	18.07	3.72	13.71 - 22.87
Intermediate (inMono)	6	1.38	0.47	0.93 - 2.11
Non-Classical (ncMono)	6	0.76	0.36	0.33 - 1.25
Dendritic Cells	6	1.29	0.43	0.72 - 1.83
Classical (cDC)	6	0.87	0.27	0.6 - 1.35
Type 1 (cDC1)	6	0.02	0.01	0.01 - 0.02
Type 2 (cDC2)	6	0.48	0.20	0.22 - 0.7
Plasmacytoid (pDC)	6	0.40	0.23	0.1 - 0.77
Transitional (tDC)	6	0.02	0.02	0 - 0.05

8.6 Summary

Teiko Bio's 25-Marker Spectral Flow Pan-Immune Profiling Test was built on the Cytex® 25-Color Immunoprofiling Kit and validated on human whole blood samples. The assay was able to detect 146 immune populations and the functional subsets within T cells, B cells, NK cells, monocytes, dendritic cells and granulocytes. The acceptance criteria for accuracy, precision within runs (intra-run), and precision between runs (inter-run) and stability were established as follow:

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Precision Assay	Intra-run	Inter-run
Benchmark	≤20%	≤25%
Median % CV (Phenotypic Cell Populations)	3.38%	4.56%
Mean % CV (Phenotypic Cell Populations)	5.19%	7.92%
% of population passing	98.2%	95.5%
Median % CV (Functional Marker Subsets)	5.50%	7.28%
Mean % CV (Functional Marker Subsets)	7.90%	12.32%
% of population passing	93.5%	90.9%

Stability Assay	1 month
Benchmark	≤25%
Median % change (Phenotypic Cell Populations)	6.51%
% of population passing	90.4%
Median % change (Functional Marker Subsets)	8.36%
% of population passing	80.4%

The assay has met CLIA's acceptance criteria and is qualified for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

9. References

Standard Operating Procedures

- DCS034 Quality Assurance and Performance Verification
- DCS124 Cytek® Aurora Operations and Maintenance
- DCS126 Cytek® data acquisition on SpectraFlo
- DCS138 Cytek 25-Color Whole Blood Immunoprofiling assay

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.

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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	Kristina Magee	2024.12.6	Initial release
02	Li-Chun Cheng	2025.01.21	Removed accelerated stability, added mean %CV to the summary of precision assay.

Reviewed by

Name	Date	Signature
Li-Chun Cheng	2024.12.6	
Jacek Klepacki	01/22/2025	