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ABC Biopharma’s Custom

[Sample Type] Cytometry Assay

Qualification Plan and Report

Project Code: ABC001

[Date Year]

Teiko Bio, Inc.

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USA

1. Table of Contents

2. Compliance Statement

The Pan-Immune Profiling mass cytometry assay for each customer project follows the qualification study plan detailed in this document. Any deviations from this plan or relevant SOPs will be documented and referenced separately. The method complies with the guidelines in DCS130 CyTOF TokuKit Performance Validation Plan and DCS034 Quality Assurance and Performance Verification but does not meet FDA regulatory requirements for in vitro diagnostic device approval (e.g., 510(k), PMA).

3. Purpose

The purpose of this Assay Performance Plan and Report document is to outline the procedures and criteria for evaluating the performance of the assay in accordance with established protocols. It ensures consistent methodology, accuracy, and reliability in results across projects while providing a clear framework for documenting any deviations or findings. This document serves as a reference for maintaining quality control and performance qualification throughout the assay process.

4. Scope

The scope of this study is to demonstrate the robustness of the laboratory developed Pan-Immune Profiling Test, based on mass cytometry, to measure immune subsets from whole blood collected from human subjects using Teiko's K2EDTA, Stable-Lyse Stable-Store TokuKit, hereby referred to as TokuKit. This document describes the following performance qualification parameters and results:

- Accuracy
- Precision
- Reference Ranges

The test results in this document are provided by Teiko Bio's CLIA laboratory located at 675 S Arapeen Dr. Suite 301, Salt Lake City, Utah 84108.

5. Responsibility

The Laboratory Director 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.

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

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

6. Acronyms

Acronym	Definition
CV	Coefficient of variation
EDTA	Ethylenediaminetetraacetic acid
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
TokuKit	Teiko's blood collection kit using K2EDTA anticoagulant and Stable-Lyse, Stable-Store as fixative

7. Definitions

Term	Definition
Performance qualification	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 precision of ABC001 Pan-Immune Profiling assay for the analysis of major immune subpopulations for granulocytes, T cells, B cells, NK cells and myeloid cells in whole blood prepared for mass cytometry with Teiko's TokuKit.

8.2 Assay Summary

Mass cytometry, also known as CyTOF (Cytometry by Time of Flight), is a versatile single-cell mass spectrometry platform that is valuable across all stages of drug development and clinical applications. It profiles immune cell subsets from various tissues by detecting cell surface or intracellular antigens related to the immune system. These antigens are stained with monoclonal antibodies conjugated to rare-earth metals, which are then detected at the single-cell level using a modified mass spectrometer after the cells are disintegrated by a plasma torch. Mass cytometry enables the use of panels with up to 45 markers, with minimal signal spillover between channels.

Table 1: ABC001 assay details

Feature	**Details**
Assay Name	ABC Biopharma's Custom Whole Blood Mass Cytometry Assay (ABC001)
Method	Mass cytometry (CyTOF)
Number of Markers	42
Instrument	Helios mass cytometer (Standard BioTools, South San Francisco, CA, USA)
Sample Type	Human whole blood
Collection Method	TokuKit
Fixation Buffer	Stable-Lyse and Stable-Store (Smart Tube Inc, Las Vegas, NV, USA)
Anticoagulant for Vacutainer	K2EDTA
Controls	N/A for qualification study. Each CyTOF run of clinical samples will include two controls, consisting of different proportions of healthy whole blood and ex vivo stimulated PBMCs. These controls will assess panel performance, detect batch effects, and aid in batch normalization.
Calibration Reagents	EQ4 Element Calibration Beads (Standard BioTools)
Software	CyTOF Software (Standard BioTools, South San Francisco, CA, USA) CellEngine (CellCarta, Montreal, Canada) RStudio Desktop 2023.06.1+524 (Posit, Boston, Massachusetts, USA) Premessa R script 0.3.4 on GitHub (San Francisco, California, USA)

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8.3 Materials and Methods

Table 2: List of critical reagents

Material	Manufacturer	Catalog #
Stable-Lyse V2	SmartTube Inc	501351694
Stable-Store V2	SmartTube Inc	501351693
K2EDTA Vacutainers	BD Biosciences	367861
15-mL HDPE Diagnostic Bottles with Closure	Thermo Scientific Nalgene	3220029050
Transfer Pipettes	Falcon	357524
Trypan Blue Dye 0.4%	Thermo Scientific	T10282
Countess™ Cell Counting Slide Chamber	Thermo Scientific	C10228
10X Phosphate-Buffered Saline (PBS)	Fisher Scientific	BP3991
High-purity Palladium Nitrate (Barcode)	Trace Sciences	Custom orders
10X Maxpar Barcode Perm (Saponin)	Standard BioTools	201057
10X eBioscience Permeabilization Buffer	Thermo Fisher	00-8333-56
Human TruStain FcX	BioLegend	422302
500 uM Iridium Solution	Standard BioTools	201192B
16% Paraformaldehyde Aqueous Solution	Electron Microscopy Sciences	15710S
Maxpar CAS Plus	Standard BioTools	201244
0.5M EDTA, pH 8.0 (100X)	Thermo Fisher	15575020
30% BSA	Sigma Aldrich	A728450ML
EQ Four Element Calibration Beads	Standard BioTools	201078
Dimethylsulfoxide (DMSO)	Sigma	D2650

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

Table 3. List of antibodies

Channel	Metal	Protein	Clone	Manufacturer	Catalog #	Lot #	Optimal Concentration (µg/mL)
89	Y	A	1	Company A	637335	MAB098	1.5
111	Cd	B	2	Company A	669735	MAB287	0.1875
111	CS	C	3	Company A	639935	MAB286	0.1875
112	Cd	D	4	Company A	677035	MAB315	0.75
113	In	E	5	Company A	636761	MAB177	3
114	Cd	F	6	Company A	630935	MAB109	0.375
115	In	G	7	Company A	638435	MAB099	0.75
116	Cd	H	8	Company A	634635	MAB110	3

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141	Pr	I	9	Company A	633761	MAB173	0.75
142	Nd	J	10	Company A	635535	MAB127	3
143	Nd	K	11	Company A	646535	MAB305	0.1875
144	Nd	L	12	Company A	656335	MAB329	3
145	Nd	M	13	Company A	633803	MAB114	0.375
146	Nd	N	14	Company A	667971	MAB301	1.5
147	Sm	O	15	Company A	660535	MAB115	0.1875
148	Nd	P	16	Company A	634195	MAB334	3
149	Sm	Q	17	Company D	888682	MAB335	0.75
150	Nd	R	18	Company A	638735	MAB116	0.75
151	Eu	S	19	Company A	639335	MAB129	0.375
152	Sm	T	20	Company A	664535	MAB130	1.5
153	Eu	U	21	Company A	637435	MAB077	0.75
154	Sm	V	22	Company A	678335	MAB131	1.5
156	Gd	W	23	Company A	652035	MAB071	1.5
157	Gd	X	24	Company A	670435	MAB306	3
158	Gd	Y	25	Company B	6481343B	MAB133	0.1875
159	Tb	Z	26	Company C	5536BF	MAB328	0.375
160	Gd	A	27	Company A	977135	MAB080	1.5
161	Dy	B	28	Company B	6494337B	MAB072	0.75
162	Dy	C	29	Company B	6495344A	MAB019	3
164	Dy	D	30	Company A	662235	MAB082	3
165	Ho	E	31	Novus Biologicals	DDX3033P433	MAB170	0.375
166	Er	F	32	Company A	659135	MAB085	0.75
167	Er	G	33	Company B	6490332A	MAB380	0.75
168	Er	H	34	Company A	683835	MAB291	0.75
169	Tm	I	35	Company A	689435	MAB045	1.5
170	Er	J	36	Company A	681935	MAB074	0.1875
171	Yb	K	37	Thermo Fisher	49-2833-15	MAB330	0.375
172	Yb	L	38	Company A	636835	MAB089	1.5
173	Yb	M	39	Proteintech	90753-4-Ig	MAB371	1.5
174	Yb	N	40	Company A	652235	MAB122	1.5
175	Lu	O	41	Company A	692635	MAB076	0.375
176	Yb	P	42	Company D	882376	MAB090	0.1875
209	Bi	Q	43	Company A	635383	MAB269	3

Table 4: List of instruments to be used for qualification experiments

Instrument	Manufacturer	Serial #	Purpose	Last Calibrated	Calibration Frequency
Helios #1	Standard BioTools	1070010393C	Mass cytometer	9/12/2024	Annual
Helios #2	Standard BioTools	1070011428C	Mass cytometer	10/14/2024	Annual

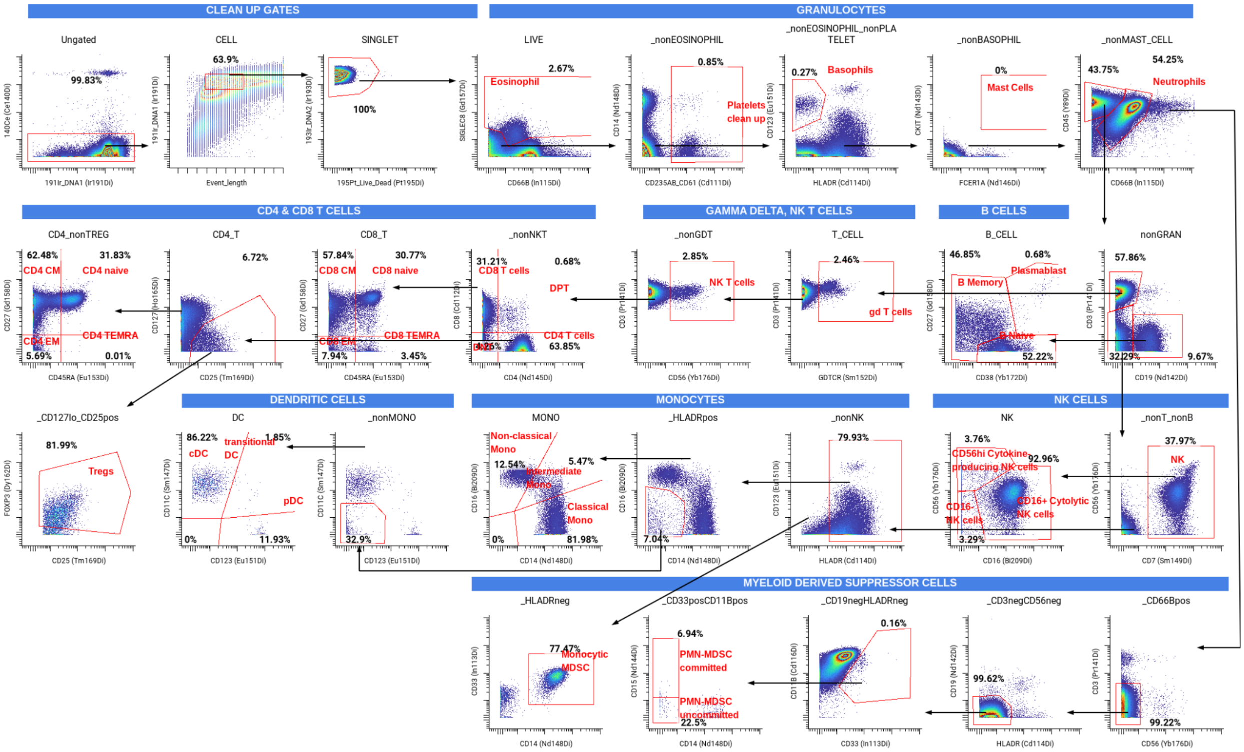
All other equipment not listed here (pipette, biosafety cabinets, centrifuges, fume hoods, etc.) are calibrated on an annual basis and will be within the calibration expiration date at the time of the planned qualification experiments.

Table 5: Pan-Immune Profiling cell subset definitions

Cell Subset	Definition (Marker Parameters)
Top level gate: Leukocyte (gating starts from non-platelet/erythrocyte)	
Eosinophil	Siglec8+ CD66b+
Basophil	CD61- CD235a- CD123+ HLA-DR- non-eosinophil
Mast Cell	cKit+ FcER1+ non-basophil
Neutrophil	CD66b+ non-mast cell
Polymorphonuclear myeloid-derived suppressor cell (PMN-MDSC)	CD66b+ CD3- CD56- HLA-DR- CD33+ CD14-
PMN-MDSC (CD15+)	CD66b+ CD3- CD56- HLA-DR- CD33+ CD14- CD15+
PMN-MDSC (CD15-)	CD66b+ CD3- CD56- HLA-DR- CD33+ CD14- CD15-
Top level gate: Non-Granulocyte (gating starts from CD45+ non-neutrophil)	
B cell	CD19+ CD3- CD14- CD33-
Naive	CD19+ CD3- CD14- CD33- CD38+ CD27-
Memory	CD19+ CD3- CD14- CD33- CD38-
Plasmablast (PB)	CD19+ CD3- CD14- CD33- CD38hi CD27+
T cell	CD3+ CD19- CD14- CD33-
Gamma Delta (γδ) T cell	CD3+ CD19- CD14- CD33- gdTCR+
Natural Killer T cell (NKT)	CD3+ CD19- CD14- CD33- gdTCR- CD56+
CD4/CD8 Double-Negative T cell (DNT)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8-
CD4/CD8 Double-Positive T cell (DPT)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8+
CD4+ T cell	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8-
Regulatory T cell (Treg)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD127lo CD25+ FOXP3+
Naive	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD25- CD45RA+ CD27+
Central Memory (TCM)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD25- CD45RA- CD27+
Effector Memory (TEM)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8-

	CD25- CD45RA- CD27-
CD45RA+ Effector Memory (TEMRA)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4+ CD8- CD25- CD45RA+ CD27-
CD8+ T cell	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+
Naive	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+ CD25- CD45RA+ CD27+
Central Memory (TCM)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+ CD25- CD45RA- CD27+
Effector Memory (TEM)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+ CD25- CD45RA- CD27-
CD45RA+ Effector Memory (TEMRA)	CD3+ CD19- CD14- CD33- gdTCR- CD56- CD4- CD8+ CD25- CD45RA+ CD27-
Natural Killer (NK) cell	CD3- CD19- CD14- CD33- CD56+ CD7+
CD16+	CD3- CD19- CD14- CD33- CD7+ CD56+ CD16+
CD16-	CD3- CD19- CD14- CD33- CD7+ CD56+ CD16-
CD56+	CD3- CD19- CD14- CD33- CD7+ CD56hi CD16-
Dendritic Cell	HLA-DR- CD14- CD16- CD123+ OR CD11c+ non-NK cell
Classical (cDC)	HLA-DR- CD14- CD16- CD123- CD11c+ non-NK cell
Plasmacytoid (pDC)	HLA-DR- CD14- CD16- CD123+ CD11c- non-NK cell
Transitional (tDC)	HLA-DR- CD14- CD16- CD123+ CD11c+ non-NK cell
Monocyte	HLA-DR+ CD14+ OR CD16+ non-NK cell
Classical (cMono)	HLA-DR+ CD14+ CD16- non-NK cell
Intermediate (inMono)	HLA-DR+ CD14+ CD16+ non-NK cell
Non-Classical (ncMono)	HLA-DR+ CD14- CD16+ non-NK cell
Monocyte myeloid-derived suppressor cell (MoMDSC)	HLA-DR- CD14+ CD33+ non-NK cell

Figure 1: Gating strategy used for ABC001 assay



1. Sample collection

For the qualification study, whole blood from three healthy donors was collected in 10-mL K2EDTA vacutainers and processed within 2 hours using the Stable-Lyse and Stable-Store buffers, following the manufacturer's instructions. Briefly, 2 mL of blood was mixed thoroughly with 2.8 mL of Stable-Lyse and incubated for 15 minutes at room temperature (RT), followed by the addition of 8 mL of Stable-Store to quench the reaction. The processed blood was then stored at -80°C until antibody staining.

For a prospective clinical study, whole blood from study subjects will be processed using TokuKit, frozen, and shipped on dry ice. Upon arrival at Teiko's laboratory, each sample will be assessed for the following:

- Possible leakage or contamination
- Thawing during storage
- Wrong or outdated specimen container, fixative, preservative or transport media
- Compromised quality at or beyond ability to complete testing. This can include:
  - Contamination with clotted red blood cells
  - Total cell count < 0.7 million cells

Any samples exhibiting these issues will be rejected for use in the assay.

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

2. Sample barcoding

Samples were barcoded prior to antibody staining using a 20-Plex Pd Barcoding Kit following manufacturer instructions and as described in DCS007 CyTOF Staining with Barcode. Briefly, after counting, 1 million cells were washed twice with 1X Maxpar barcode perm buffer and then barcoded with unique combinations of Pd isotopes for 30 min at RT on a shaker. After barcoding, cells were washed twice with 1X eBioscience permeabilization (eBio perm) buffer, incubated for 10 minutes in the second eBio perm wash, and washed once with Cell Staining Buffer (CSB). After washes, barcoded samples were pooled into a single tube.

3. Antibody staining

Antibody staining was performed as described in DCS007 CyTOF Staining with Barcode; buffers were prepared as described in DCS006 Preparation of CyTOF reagents. An antibody cocktail was prepared in advance in CSB according to previously determined titers at 25 μL of staining volume per 1 million cells. The master mix of antibody cocktail was aliquoted into single use and then stored in -80°C until the day of staining. The pooled barcoded sample was stained for 10 min at RT with TruStain FcX receptor blocking solution followed by 1 hr incubation with antibody cocktail at RT. Cells were stained using a panel of 42 antibodies (Table 3), washed twice with CSB and resuspended in intercalation solution (4% PFA in PBS and 0.25 μM iridium intercalator) for 20 min at RT. Cells were pelleted, resuspended in a small volume of CSB supplemented with 10% DMSO and frozen at -80°C until the day of acquisition.

4. Data acquisition

Helios mass cytometer performance QC is described in DCS002 CyTOF Operation and Maintenance. Once the instrument is ready, proceed sample preparation as described in DCS007 CyTOF Staining with Barcode. Before acquisition, samples were washed twice in Maxpar CAS Plus, resuspended at 0.7 million cells/mL in CAS-Plus supplemented with EQ4 element calibration beads, and filtered through a 40 μm cell strainer. Cells were acquired at 300 to 350 events per second.

5. Data analysis

Manual gating of FCS files was performed using CellEngine. Cell populations were gated based on the parameters described in Table 5 and as shown in Figure 1. Frequencies were determined as % of top level gates. For example, granulocyte subsets will be measured as % of total leukocytes defined as live CD61- CD235a- singlets and eosinophils. Other cell lineages will be measured as % of total non-granulocytes. Gated populations with a median frequency below 100 cells were excluded from the analysis to avoid inaccurate measurements caused by low-frequency populations. Expression of functional state markers above background were gated and measured as % of parent population. Parent populations and gated functional state under 100 cells were excluded from the analysis. The median channel values (MCVs) of gated functional states are reported as arcsinh-transformed values, using a cofactor of 5.

For precision analyses, frequencies of cell populations and functional states were compared to their corresponding populations across technical replicates, 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. A minimum number of two replicates is required for the calculation of % CV.

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

8.4 Qualification Parameters

1. Assay Optimization and Accuracy

Method optimization was performed to select the optimal channels, metals, and concentrations for each marker. We were able to identify an optimal concentration for each antibody in the panel with minimal to no spillover in other channels. The optimized concentration used in this study is reported in Table 3. To confirm the panel is able to detect all major immune cell populations, accuracy was assessed by determining frequencies of all major immune cell lineages and subsets in control samples as described in the Panel Verification report.

2. Precision

Intra-run and Inter-run were evaluated to ensure test results are not affected by technical variables (e.g. operators) during the sample processing step, or by the individual mass cytometry instrument run.

a. Intra-run: each fixed blood sample was split into three technical replicates, and all replicates were barcoded, pooled and stained by the same operator, who ran all samples together on the same Helios in one day. Variance between sets of replicates was calculated for precision analysis. Acceptance criteria were ≥95% of immune measurements should have a CV ≤ 30% between replicates.
b. Inter-run: each fixed blood sample was split into three technical replicates and processed on three separate days by different operators. Variance between runs was calculated for precision analysis. Acceptance criteria were ≥ 95% of immune measurements should have ≤30% CV between individual runs.

3. Reference Range

Median, standard deviation and range was calculated for each major immune population from three healthy donors.

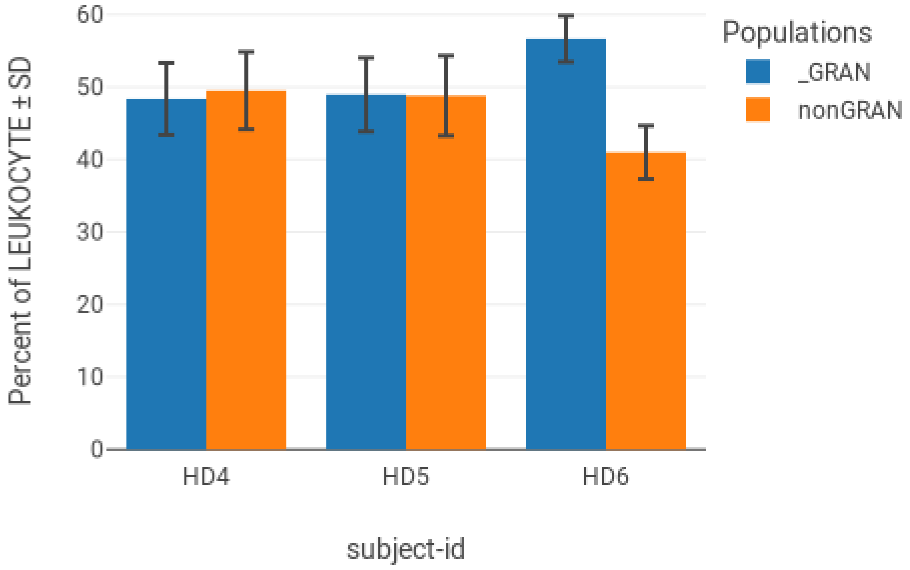
The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

8.5 Results

1. Immune Cell Frequency

Immune cell frequency is determined by dividing the number of events in the specific subpopulation gate (defined in Table 5) by the total events in the parent (top-level) gate, reported as a percentage.

Figure 2: Frequency of major immune lineages in whole blood of healthy subjects measured by ABC001 assay.



The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Figure 3: Frequency of major immune cell types in granulocytes of healthy subjects measured by ABC001 assay.

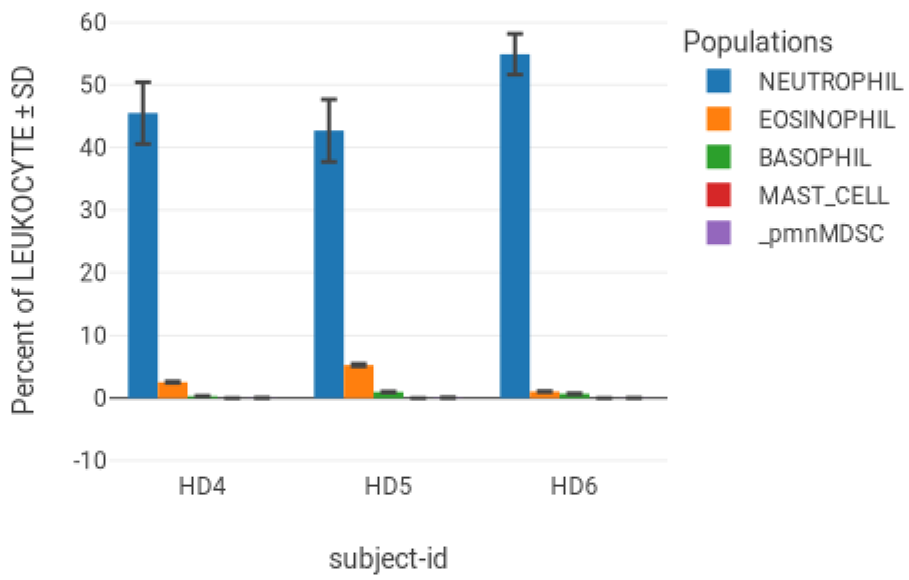
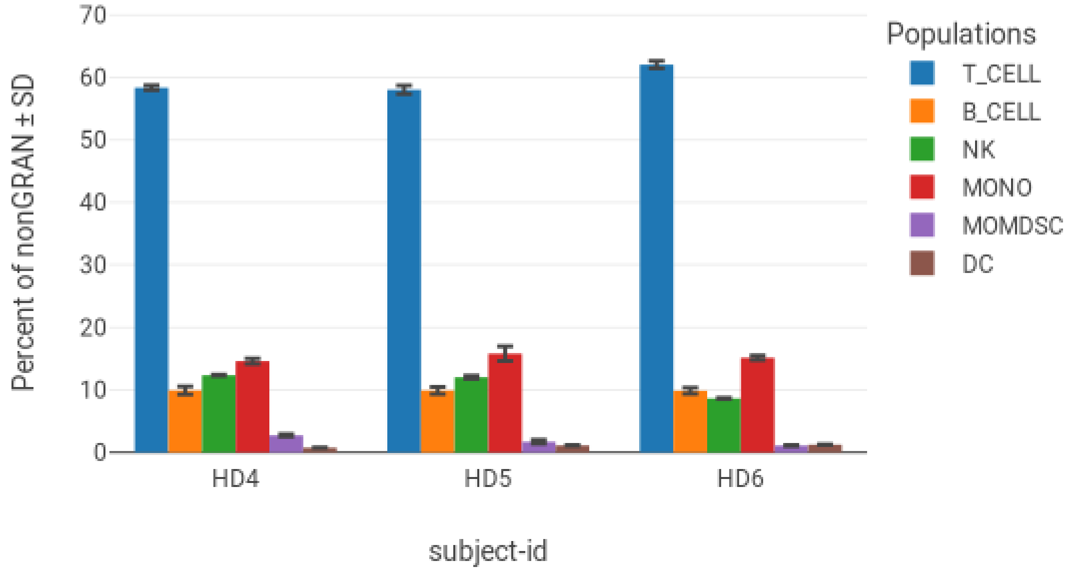


Figure 4: Frequency of major immune cell types in non-granulocytes of healthy subjects measured by ABC001 assay.



The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

2. Intra-run Precision of Immune Cell Frequency

Coefficient of variation between three replicates of the same run was calculated per subject for the intra-run precision analysis and is reported in Table 6-8 and Figure 5. All measurable cell populations passed the acceptable threshold of ≤ 30% variation between replicates. **The median of intra-run %CV for all measurable populations across all three healthy subjects was 1.64%**, with individual median %CVs of 1.64%, 1.34%, and 2.19% for Donor 1 (HD4), Donor 2 (HD5), and Donor 3 (HD6), respectively.

Table 6: Intra-Run Precision (%CV) of Immune Cell Frequencies for Donor 1 (HD4)

Cell Subset	**# Replicates in %CV calculation**	**Median # of cells measured**	**Average % of top-level gate**	**Variation between replicates (%CV)**
Granulocyte				
Eosinophil	3	4485	2.49	4.10
Basophil	3	604	0.34	4.95
Mast Cell	0*	0	0.00	EXCLUDED
Neutrophil	3	75764	41.27	2.75
PMN-MDSC (CD15+)	0*	3	0.00	EXCLUDED
PMN-MDSC (CD15-)	0*	39	0.02	EXCLUDED
Non-Granulocyte				
B cell	3	10285	10.46	1.63
Naive	3	5427	5.52	1.83
Memory	3	4771	4.84	1.38
PB	0*	70	0.07	EXCLUDED
T cell	3	57113	58.52	0.57
γδT	3	1478	1.50	0.53
NKT	3	1712	1.80	2.83
DNT	3	1653	1.74	2.81
DPT	3	422	0.42	1.51
CD4+ T cell	3	34837	35.76	0.90
Treg	3	2251	2.27	3.51
Naive	3	10429	10.70	1.74
TCM	3	20096	20.64	0.74
TEM	3	1720	1.79	3.57
TEMRA	0*	0	0.00	EXCLUDED
CD8+ T cell	3	17011	17.29	0.33
Naive	3	5408	5.51	1.36
TCM	3	9680	9.84	0.45

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

TEM	3	1308	1.32	1.36
TEMRA	3	615	0.62	1.41
NK cell	3	12055	12.33	0.49
CD16+	3	11383	11.62	0.60
CD16-	3	275	0.28	6.37
CD56 high	3	418	0.44	2.90
Dendritic Cell	3	678	0.71	2.95
cDC	3	589	0.61	1.82
pDC	1*	86	0.09	EXCLUDED
tDC	0*	8	0.01	EXCLUDED
Monocytes	3	14163	14.28	1.15
cMono	3	11805	11.88	1.29
inMono	3	682	0.71	1.65
ncMono	3	1676	1.68	1.34
MoMDSCs	3	2549	2.62	3.77
*Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations.			**Donor 1 (HD4) Median**	**1.57**

Table 7: Intra-Run Precision (%CV) of Immune Cell Frequencies for Donor 2 (HD5)

Cell Subset	**# Replicates in %CV calculation**	**Median # of cells measured**	**Average % of top-level gate**	**Variation between replicates (%CV)**
Granulocyte				
Eosinophil	3	10690	5.14	0.13
Basophil	3	2126	1.03	1.26
Mast Cell	0*	0	0.00	EXCLUDED
Neutrophil	3	80390	38.22	0.95
PMN-MDSC (CD15+)	0*	31	0.01	EXCLUDED
PMN-MDSC (CD15-)	2	102	0.05	16.29
Non-Granulocyte				
B cell	3	11593	10.41	0.34
Naive	3	8631	7.74	0.88
Memory	3	2953	2.63	1.33
PB	0*	30	0.03	EXCLUDED
T cell	3	64975	58.43	0.09
γδT	3	3124	2.78	1.23
NKT	3	887	0.81	1.35
DNT	3	2838	2.51	1.54
DPT	3	161	0.15	7.54

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

CD4+ T cell	3	39708	35.58	0.51
Treg	3	2289	2.01	2.50
Naive	3	8170	7.34	0.50
TCM	3	26538	23.74	0.65
TEM	3	2301	2.09	1.71
TEMRA	0*	2	0.00	EXCLUDED
CD8+ T cell	3	18560	16.60	0.90
Naive	3	6907	6.22	0.38
TCM	3	10607	9.44	1.36
TEM	3	942	0.84	3.00
TEMRA	2	110	0.10	21.38
NK cell	3	13197	11.88	0.19
CD16+	3	11159	10.00	0.37
CD16-	3	962	0.87	7.74
CD56 high	3	1090	0.99	6.74
Dendritic Cell	3	1199	1.07	1.81
cDC	3	797	0.73	3.71
pDC	3	355	0.31	6.38
tDC	0*	30	0.03	EXCLUDED
Monocytes	3	16625	14.85	1.05
cMono	3	14145	12.64	0.94
inMono	3	874	0.78	0.60
ncMono	3	1590	1.42	2.76
MoMDSCs	3	2141	1.93	5.23
*Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations.			**Donor 2 (HD5) Median**	**1.29**

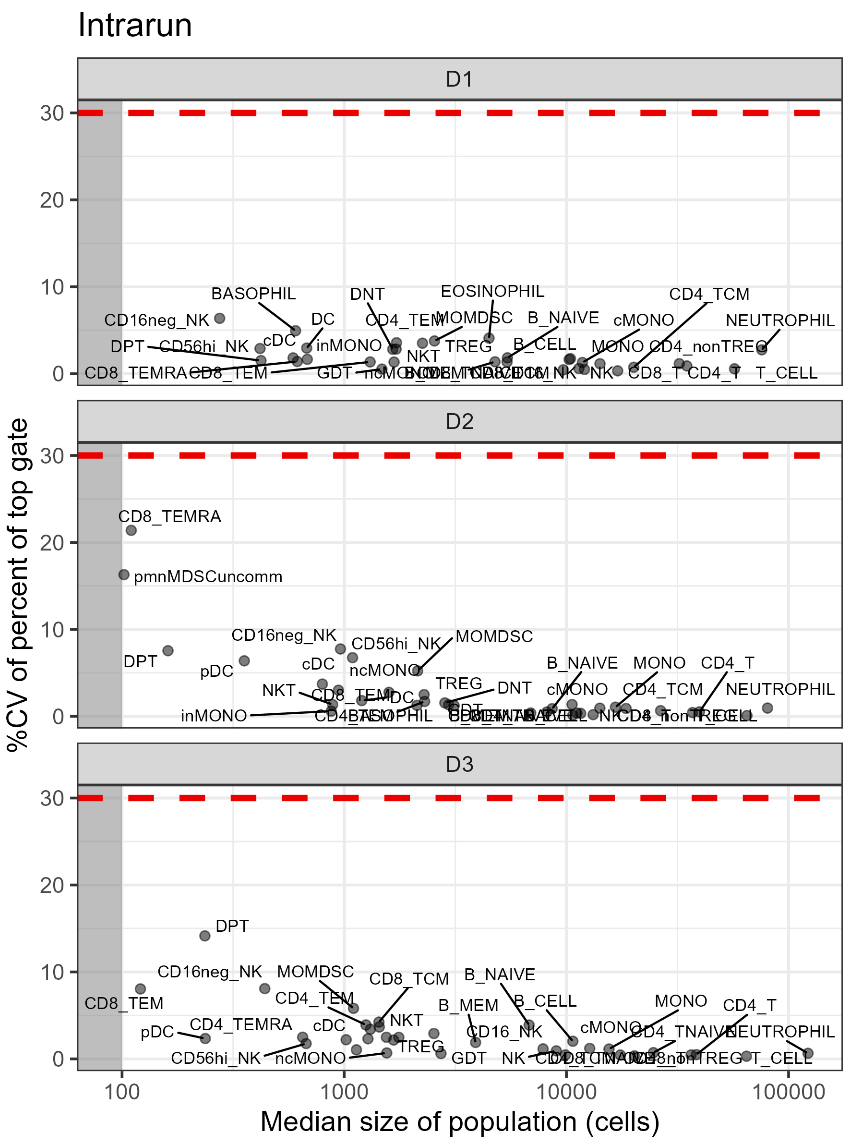
Table 8: Intra-Run Precision (%CV) of Immune Cell Frequencies for Donor 3 (HD6)

Cell Subset	**# Replicates in %CV calculation**	**Median # of cells measured**	**Average % of top-level gate**	**Variation between replicates (%CV)**
Granulocyte				
Eosinophil	3	2533	1.05	2.92
Basophil	3	1761	0.75	2.48
Mast Cell	0*	1	0.00	EXCLUDED
Neutrophil	3	122283	52.04	0.64
PMN-MDSC (CD15+)	0*	15	0.01	EXCLUDED
PMN-MDSC (CD15-)	0*	57	0.02	EXCLUDED
Non-Granulocyte				

B cell	3	10691	10.26	2.03
Naive	3	6794	6.50	3.89
Memory	3	3902	3.70	1.89
PB	0*	50	0.05	EXCLUDED
T cell	3	64636	61.82	0.31
γδT	3	2726	2.62	0.62
NKT	3	1548	1.46	2.48
DNT	3	1438	1.35	3.67
DPT	3	236	0.24	14.15
CD4+ T cell	3	38457	36.88	0.49
Treg	3	1670	1.59	2.17
Naive	3	24639	23.69	0.72
TCM	3	9894	9.40	0.46
TEM	3	1253	1.20	3.90
TEMRA	3	650	0.62	2.49
CD8+ T cell	3	20244	19.27	0.35
Naive	3	17487	16.72	0.45
TCM	3	1433	1.35	4.23
TEM	3	121	0.12	8.05
TEMRA	3	1135	1.07	1.04
NK cell	3	9008	8.56	0.92
CD16+	3	7851	7.50	1.15
CD16-	3	439	0.41	8.08
CD56 high	3	674	0.64	1.76
Dendritic Cell	3	1279	1.22	2.31
cDC	3	1020	0.97	2.20
pDC	3	237	0.23	2.34
tDC	0*	22	0.02	EXCLUDED
Monocytes	3	15550	14.88	1.13
cMono	3	12735	12.18	1.20
inMono	3	1312	1.23	3.41
ncMono	3	1554	1.47	0.69
MoMDSCs	3	1101	1.04	5.81
*Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations.			**Donor 3 (HD6) Median**	**2.10**

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Figure 5: Coefficient of Variation in Frequency (Percentage of Top Gate) and Median Number of Cells per Immune Population for Intra-Run Precision Assessment



3. Inter-Run Precision of Immune Cell Frequency

Coefficient of variation between three replicates of three runs was calculated per subject for the inter-run precision analysis and is reported in Table 9-11 and Figure 6. All measurable cell populations passed the acceptable threshold of ≤ 30% variation between replicates. **The median of inter-run %CV for all measurable populations across all three healthy subjects was 3.69%**, with individual median %CVs of 3.61%, 3.49%, and 3.81% for Donor 1 (HD4), Donor 2 (HD5), and Donor 3 (HD6), respectively.

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Table 9: Inter-Run Precision (%CV) of Immune Cell Frequencies for Donor 1 (HD4)

Cell Subset	**# Replicates in %CV calculation**	**Median # of cells measured**	**Average % of top-level gate**	**Variation between replicates (%CV)**
Granulocyte				
Eosinophil	3	9718	2.59	5.22
Basophil	3	1117	0.27	2.64
Mast Cell	0*	0	0.00	EXCLUDED
Neutrophil	3	198352	49.75	4.86
PMN-MDSC (CD15+)	0*	23	0.01	EXCLUDED
PMN-MDSC (CD15-)	1*	81	0.04	EXCLUDED
Non-Granulocyte				
B cell	3	17133	9.41	4.37
Naive	3	8947	4.89	3.08
Memory	3	8026	4.42	5.65
PB	2	117	0.07	29.02
T cell	3	103192	58.21	0.75
γδT	3	2542	1.48	4.29
NKT	3	2867	1.75	7.80
DNT	3	4169	2.36	1.14
DPT	3	663	0.37	1.21
CD4+ T cell	3	62433	34.99	0.22
Treg	3	3442	1.91	2.44
Naive	3	18534	10.77	4.43
TCM	3	36383	20.11	1.84
TEM	3	3127	1.78	4.30
TEMRA	0*	3	0.00	EXCLUDED
CD8+ T cell	3	30518	17.26	1.59
Naive	3	9389	5.40	2.53
TCM	3	17653	9.90	0.83
TEM	3	2424	1.37	2.51
TEMRA	3	968	0.59	9.71
NK cell	3	21860	12.39	1.25
CD16+	3	20322	11.54	1.43
CD16-	3	666	0.38	5.59
CD56 high	3	823	0.48	3.23
Dendritic Cell	3	1350	0.78	3.69
cDC	3	1164	0.68	4.20

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

pDC	3	161	0.09	3.08
tDC	0*	22	0.01	EXCLUDED
Monocytes	3	26580	14.97	1.93
cMono	3	21791	12.43	3.53
inMono	3	1166	0.76	14.21
ncMono	3	3150	1.77	4.56
MoMDSCs	3	4892	2.84	8.54
*Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations.			**Donor 1 (HD4) Median**	**3.38**

Table 10: Inter-Run Precision (%CV) of Immune Cell Frequencies for Donor 2 (HD5)

Cell Subset	**# Replicates in %CV calculation**	**Median # of cells measured**	**Average % of top-level gate**	**Variation between replicates (%CV)**
Granulocyte				
Eosinophil	3	21833	5.42	3.49
Basophil	3	3660	0.91	3.14
Mast Cell	0*	0	0.00	EXCLUDED
Neutrophil	3	197193	47.18	2.82
PMN-MDSC (CD15+)	0*	27	0.01	EXCLUDED
PMN-MDSC (CD15-)	1*	92	0.06	EXCLUDED
Non-Granulocyte				
B cell	3	17142	9.43	3.37
Naive	3	12132	6.77	1.92
Memory	3	4879	2.60	7.27
PB	0*	66	0.04	EXCLUDED
T cell	3	103943	57.64	1.44
γδT	3	5073	2.77	2.47
NKT	3	1278	0.76	18.99
DNT	3	5511	3.13	2.63
DPT	3	264	0.15	13.19
CD4+ T cell	3	62730	34.68	1.30
Treg	3	3328	1.73	7.10
Naive	3	12882	7.31	5.35
TCM	3	41913	23.07	1.24
TEM	3	3821	2.10	1.99
TEMRA	0*	7	0.00	EXCLUDED
CD8+ T cell	3	28699	16.14	2.04
Naive	3	10631	5.99	2.45

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

TCM	3	16537	9.27	1.69
TEM	3	1373	0.79	3.05
TEMRA	3	158	0.09	13.70
NK cell	3	21560	12.19	2.50
CD16+	3	16786	9.38	2.99
CD16-	3	2784	1.72	8.56
CD56 high	3	1945	1.08	3.37
Dendritic Cell	3	1928	1.15	6.00
cDC	3	1403	0.83	5.18
pDC	3	496	0.30	8.99
tDC	0*	29	0.02	EXCLUDED
Monocytes	3	30272	16.81	4.52
cMono	3	26374	14.35	5.45
inMono	3	1389	0.94	17.42
ncMono	3	2553	1.51	5.79
MoMDSCs	3	2359	1.47	19.57
*Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations.			**Donor 2 (HD5) Median**	**3.37**

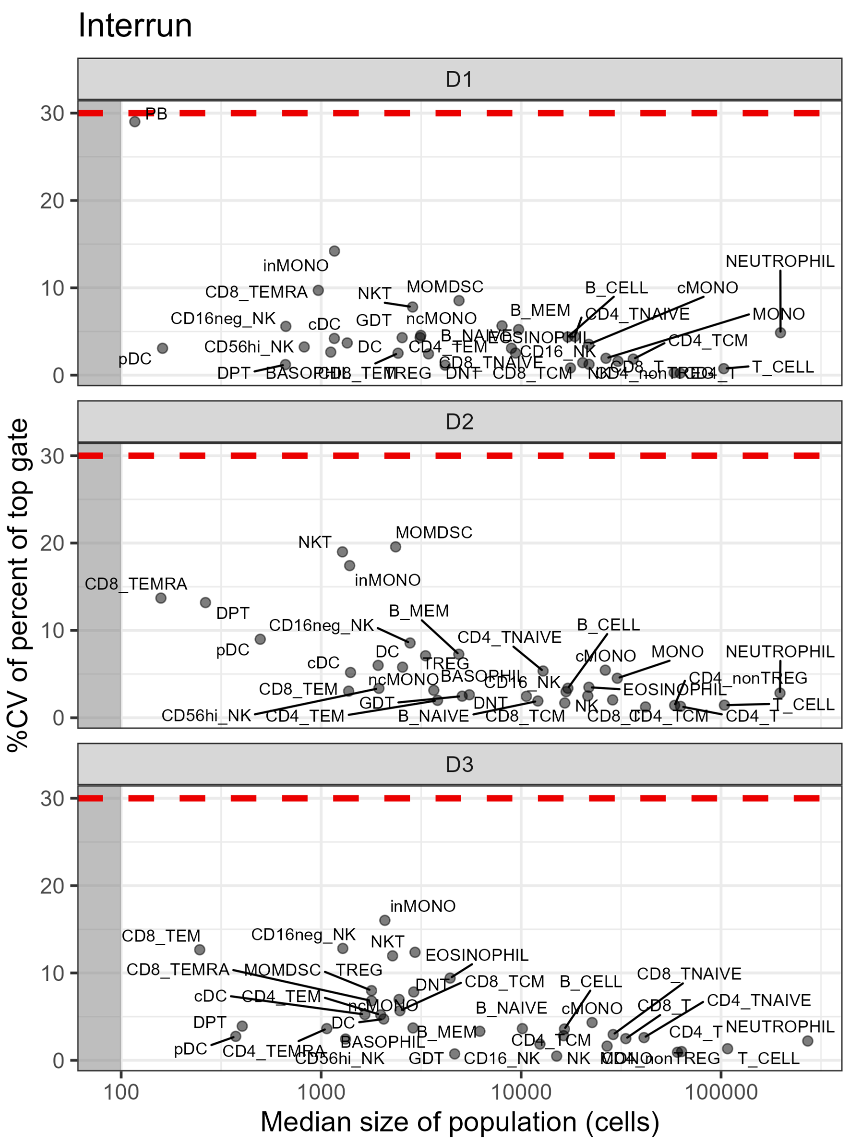
Table 11: Inter-Run Precision (%CV) of Immune Cell Frequencies for Donor 3 (HD6)

Cell Subset	**# Replicates in %CV calculation**	**Median # of cells measured**	**Average % of top-level gate**	**Variation between replicates (%CV)**
Granulocyte				
Eosinophil	3	4417	1.01	9.41
Basophil	3	2880	0.63	3.71
Mast Cell	0*	1	0.00	EXCLUDED
Neutrophil	3	271496	57.81	2.21
PMN-MDSC (CD15+)	0*	15	0.00	EXCLUDED
PMN-MDSC (CD15-)	1*	45	0.02	EXCLUDED
Non-Granulocyte				
B cell	3	16479	9.49	3.59
Naive	3	10147	5.88	3.63
Memory	3	6219	3.55	3.32
PB	1*	92	0.05	EXCLUDED
T cell	3	107849	62.29	1.33
γδT	3	4653	2.68	0.73
NKT	3	2948	1.53	12.37
DNT	3	2904	1.85	7.82

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

DPT	3	403	0.23	3.91
CD4+ T cell	3	63508	36.75	1.00
Treg	3	2276	1.21	11.96
Naive	3	41193	23.98	2.60
TCM	3	16309	9.41	2.82
TEM	3	1985	1.16	5.20
TEMRA	3	1067	0.63	3.63
CD8+ T cell	3	33387	19.24	2.48
Naive	3	28877	16.53	2.96
TCM	3	2477	1.53	5.68
TEM	3	247	0.15	12.66
TEMRA	3	1786	1.02	6.81
NK cell	3	15062	8.72	0.48
CD16+	3	12420	7.09	1.86
CD16-	3	1283	0.86	12.81
CD56 high	3	1324	0.75	2.44
Dendritic Cell	3	2056	1.26	4.73
cDC	3	1654	1.02	5.25
pDC	3	374	0.22	2.74
tDC	0*	28	0.02	EXCLUDED
Monocytes	3	26917	15.44	1.63
cMono	3	22652	12.62	4.33
inMono	3	2083	1.27	16.02
ncMono	3	2453	1.55	6.97
MoMDSCs	3	1787	1.14	7.98
*Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations.			**Donor 3 (HD6) Median**	**3.67**

Figure 6: Coefficient of Variation in Frequency (Percentage of Top Gate) and Median Number of Cells per Immune Population for Inter-Run Precision Assessment



4. Reference Range of Immune Cell Frequency

Table 12: Reference Range of Healthy Subjects Processed with ABC001 assay.

		% of Leukocytes / Non-Granulocytes		
Cell Subset	**# Subjects**	**Median**	**Standard Deviation**	**Range**
Granulocyte				

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Eosinophil	3	2.59	2.23	1.01 - 5.42
Basophil	3	0.63	0.32	0.27 - 0.63
Mast Cell	0*	EXCLUDED	EXCLUDED	EXCLUDED
Neutrophil	3	49.75	5.54	47.18 - 57.81
PMN-MDSC (CD15+)	0	0.01	0.00	EXCLUDED
PMN-MDSC (CD15-)	0	0.04	0.02	EXCLUDED
Non-Granulocyte				
B cell	3	9.43	0.04	9.41 - 9.49
Naive	3	5.88	0.94	4.89 - 6.77
Memory	3	3.55	0.91	2.60 - 4.42
PB	1*	EXCLUDED	EXCLUDED	EXCLUDED
T cell	3	58.21	2.54	57.64 - 62.29
γδT	3	2.68	0.72	1.48 - 2.77
NKT	3	1.53	0.52	0.76 - 1.75
DNT	3	2.36	0.64	1.85 - 3.13
DPT	3	0.23	0.11	0.15 - 0.37
CD4+ T cell	3	34.99	1.12	34.68 - 36.75
Treg	3	1.73	0.36	1.21 - 1.91
Naive	3	10.77	8.80	7.31 - 23.98
TCM	3	20.11	7.19	9.41 - 23.07
TEM	3	1.78	0.48	1.16 - 2.10
TEMRA	1*	EXCLUDED	EXCLUDED	EXCLUDED
CD8+ T cell	3	17.26	1.57	16.14 - 19.24
Naive	3	5.99	6.27	5.40 - 16.53
TCM	3	9.27	4.66	1.53 - 9.90
TEM	3	0.79	0.61	0.15 - 1.37
TEMRA	3	0.59	0.46	0.09 - 1.02
NK cell	3	1.53	0.52	0.76 - 1.75
CD16+	3	9.38	2.23	7.09 - 11.54
CD16-	3	0.86	0.68	0.38 - 1.72
CD56 high	3	0.75	0.30	0.48 - 1.08
Dendritic Cell	3	1.15	0.25	0.78 - 1.26
cDC	3	0.83	0.17	0.83 - 1.02
pDC	3	0.22	0.11	0.09 - 0.30
tDC	0*	EXCLUDED	EXCLUDED	EXCLUDED
Monocytes	3	15.44	0.96	14.97 - 16.81
cMono	3	12.62	1.06	12.43 - 14.35

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

inMono	3	0.94	0.26	0.76 - 1.27
ncMono	3	1.55	0.14	1.51 - 1.77
MoMDSCs	3	1.47	0.90	1.14 - 2.84
*Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from range calculations.				

5. Functional State Marker

Functional state 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. The median channel value (MCV) of the functional state marker among the gated events is reported as an arcsinh-transformed value, using a cofactor of 5. See Table 13 for the list of functional state markers assessed in each immune cell population.

Table 13: Functional State 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*
CCR7	gated					
CD11B	gated	gated	gated	gated	gated	gated
CD15					gated	gated
CD161	gated		gated			
CD25		gated				gated
CD38	gated		gated			
CD38_HLADR	gated					
CD74		gated		gated	gated	
CD8			gated			
CD86	gated	gated	gated	gated	gated	
CTLA4	gated			gated	gated	
HLADR	gated	gated	gated			gated
KI67	gated	gated	gated	gated	gated	gated
LAG3	gated	gated	gated			
LOX1				gated	gated	gated
PD1	gated		gated		gated	
PDL1	gated	gated		gated	gated	gated
RBM39	gated	gated	gated	gated	gated	gated
TBET	gated	gated	gated		gated	
TCF1	gated					
TIGIT	gated		gated			
TIM3	gated		gated	gated	gated	gated

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

*Functional state markers are not assessed in eosinophils due to the non-specific binding of metal tags to eosinophil granules.

6. Intra-run Precision of Functional State Marker Frequency

Coefficient of variation of functional state marker frequency between three replicates of the same run was calculated per subject for the intra-run precision analysis and is reported in Table 14-16 and Figure 7. 99.82% (556/557) measurable cell populations passed the acceptable threshold of ≤ 30% variation between replicates. **The median of intra-run %CV for all measurable populations across all three healthy subjects was 2.77%**, with individual median %CVs of 2.96%, 1.88%, and 3.14% for Donor 1 (HD4), Donor 2 (HD5), and Donor 3 (HD6), respectively.

Table 14: Intra-Run Precision (%CV) of Functional State Marker Frequencies for Donor 1 (HD4)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD38_ HLADR	CD74	CD8	CD86	CTLA4
BASOPHIL		0.70	*		17.54						
MAST_CELL		*	*		*						
NEUTROPHIL		0.01	9.82		*						
B_CELL		1.10			2.21			2.24		*	
B_NAIVE		2.87			*			2.67		*	
B_MEM		0.81			5.87			1.75		*	
PB		*			*			*		*	
T_CELL	0.10	0.71		0.73		0.33	4.86			6.32	1.83
GDT	5.67	0.83		2.01		3.76	*			*	*
NKT	12.83	1.31		5.38		3.27	*			*	*
DNT	4.39	1.60		3.02		3.56	*			*	*
DPT	1.31	3.64		*		*	*			*	*
TREG	0.72	3.33		4.68		2.65	2.90			*	1.68
CD4_nonTREG	0.20	1.07		0.54		0.65	9.24			5.09	2.96
CD4_TNAIVE		2.32		*		0.22	*			*	*
CD4_TCM	0.28	0.71		0.58		0.93	*			*	5.12
CD4_TEM	5.16	4.48		3.71		6.72	*			*	*
CD4_TEMRA	*	*		*		*	*			*	*
CD8_T	0.24	0.48		4.40		0.42	8.43			*	*
CD8_TNAIVE		2.21		*		0.54	*			*	*
CD8_TCM	0.97	0.65		4.04		1.01	8.55			*	*
CD8_TEM	*	1.61		5.69		2.83	*			*	*
CD8_TEMRA	*	3.01		*		4.88	*			*	*
NK		0.15		0.34		0.17			2.64	2.54	
CD16_NK		0.11		0.32		0.13			2.24	1.50	
CD16neg_NK		6.69		1.21		9.21			*	*	
CD56hi_NK		0.78		2.32		0.26			*	10.79	

DC		2.02						1.33		*	*
cDC		1.69						1.73		*	*
pDC		*						*		*	*
transDC		*						*		*	*
MONO		0.12	*					9.02		4.23	*
cMONO		0.03	*					8.38		5.29	*
inMONO		0.30	*					19.64		5.11	*
ncMONO		1.11	*					13.14		0.93	*
MOMDSC		0.02	*					21.84		9.36	*
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
BASOPHIL	*	*		*		*	1.69				*
MAST_CELL	*	*		*		*	*				*
NEUTROPHIL	6.41	16.95		0.51		*	10.21				*
B_CELL	0.12	*	*			*	3.18	18.36			
B_NAIVE	0.16	*	*			*	4.31	*			
B_MEM	0.08	*	*			*	1.91	18.51			
PB	*	*	*			*	*	*			
T_CELL	4.18	16.07	*		1.85	*	2.87	18.68	1.22	2.60	*
GDT	*	*	*		*	*	3.25	16.56	5.17	4.82	*
NKT	*	*	*		*	*	1.65	11.27	7.27	2.56	*
DNT	*	*	*		11.36	*	2.73	21.38	2.04	5.88	*
DPT	*	*	*		*	*	3.51	*	1.34	*	*
TREG	4.40	*	*		*	*	3.65	*	3.20	1.69	*
CD4_nonTREG	7.08	8.36	*		5.67	*	2.81	17.99	0.32	8.90	*
CD4_TNAIVE	*	*	*		*	*	3.52	*	0.11	*	*
CD4_TCM	4.80	14.99	*		4.70	*	2.57	19.22	0.45	8.28	*
CD4_TEM	*	*	*		*	*	2.21	*	0.91	*	*
CD4_TEMRA	*	*	*		*	*	*	*	*	*	*
CD8_T	8.43	26.38	*		1.00	*	3.07	21.76	2.08	0.96	*
CD8_TNAIVE	*	*	*		*	*	3.51	11.69	0.42	4.20	*
CD8_TCM	8.01	*	*		1.39	*	2.98	23.86	2.90	1.76	*
CD8_TEM	*	*	*		3.94	*	2.10	19.91	5.24	1.91	*
CD8_TEMRA	*	*	*		*	*	2.61	19.44	8.72	4.56	*
NK	4.20	22.67	*		*		0.54	10.03		0.85	*
CD16_NK	5.17	21.33	*		*		0.60	9.97		1.19	*
CD16neg_NK	*	*	*		*		1.56	15.17		*	*
CD56hi_NK	*	*	*		*		0.76	12.72		*	*
DC		17.41		*		*	0.84				*
cDC		17.25		*		*	1.82				*
pDC		*		*		*	*				*
transDC		*		*		*	*				*

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

MONO		3.01		3.20	*	*	1.50	*			*
cMONO		3.45		5.30	*	*	1.18	*			*
inMONO		*		12.62	*	*	4.40	*			*
ncMONO		*		0.83	*	*	3.60	*			*
MOMDSC		*		6.23	*	*	4.93	*			*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

Table 15: Intra-Run Precision (%CV) of Functional State Marker Frequencies for Donor 2 (HD5)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD38_ HLADR	CD74	CD8	CD86	CTLA4
BASOPHIL		0.82	*		2.17						
MAST_CELL		*	*		*						
NEUTROPHIL		0.03	5.36		*						
B_CELL		1.26			7.64			0.37		*	
B_MEM		1.58			8.96			0.44		*	
B_NAIVE		1.26			*			0.36		*	
PB		*			*			*		*	
T_CELL	0.28	0.68		0.19		0.40	4.60			19.85	5.85
GDT	3.34	0.46		2.95		6.81	*			*	*
NKT	*	3.13		0.41		*	*			*	*
DNT	11.00	0.33		1.92		8.64	*			*	*
DPT	*	*		*		*	*			*	*
TREG	1.03	5.96		6.63		1.20	*			*	3.43
CD4_nonTREG	0.49	0.82		0.24		0.71	9.79			15.64	9.63
CD4_TCM	0.37	1.29		0.34		0.56	5.65			15.68	13.19
CD4_TEM	7.37	2.72		2.08		11.98	*			*	*
CD4_TEMRA	*	*		*		*	*			*	*
CD4_TNAIVE		1.39		*		0.32	*			*	*
CD8_T	0.40	0.81		1.19		0.47	1.57			24.65	*
CD8_TCM	0.18	1.53		1.01		1.02	1.44			*	*
CD8_TEM	*	1.24		3.02		8.97	*			*	*
CD8_TEMRA	*	*		*		*	*			*	*
CD8_TNAIVE		0.69		*		0.73	*			*	*
NK		0.23		0.25		0.11			8.64	10.73	
CD16_NK		0.25		0.16		0.25			8.30	11.49	
CD16neg_NK		1.36		1.55		1.54			*	12.35	
CD56hi_NK		0.18		3.50		0.48			*	5.28	
DC		0.93						1.05		16.97	*
cDC		1.08						1.25		15.20	*
pDC		6.05						2.15		*	*
transDC		*						*		*	*

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

MONO		0.21	*					0.64		1.70	6.65
cMONO		0.04	*					0.66		1.63	*
inMONO		0.30	*					0.65		3.81	*
ncMONO		1.70	*					3.73		1.96	7.11
MOMDSC		0.10	*					6.15		0.87	*
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
BASOPHIL	*	*		3.90		*	0.55				*
MAST_CELL	*	*		*		*	*				*
NEUTROPHIL	14.38	*		0.29		*	6.17				*
B_CELL	0.56	*	*			*	0.54	20.59			
B_MEM	0.66	*	*			*	1.27	*			
B_NAIVE	0.59	*	*			*	0.36	*			
PB	*	*	*			*	*	*			
T_CELL	10.70	3.89	*		5.04	5.22	1.38	0.51	0.63	2.20	*
GDT	*	*	*		*	*	2.03	0.39	1.03	*	*
NKT	*	*	*		*	*	1.03	2.71	1.23	*	*
DNT	*	*	*		14.26	*	1.34	5.19	0.24	5.80	*
DPT	*	*	*		*	*	5.44	*	1.42	*	*
TREG	9.29	*	*		*	*	0.41	*	1.64	2.55	*
CD4_nonTREG	13.64	3.90	*		4.18	*	1.34	3.45	0.65	2.48	*
CD4_TCM	12.53	6.29	*		4.50	*	1.49	3.36	0.62	3.03	*
CD4_TEM	*	*	*		*	*	4.02	4.12	2.77	*	*
CD4_TEMRA	*	*	*		*	*	*	*	*	*	*
CD4_TNAIVE	*	*	*		*	*	0.39	*	0.21	*	*
CD8_T	5.45	3.02	*		4.94	*	1.53	1.26	0.84	2.65	*
CD8_TCM	3.61	*	*		3.98	*	2.15	1.06	1.23	3.18	*
CD8_TEM	*	*	*		14.40	*	3.20	5.94	3.51	3.94	*
CD8_TEMRA	*	*	*		*	*	*	*	*	*	*
CD8_TNAIVE	*	*	*		*	*	0.47	3.09	0.22	6.80	*
NK	11.02	6.62	*		*		0.99	0.44		3.25	2.59
CD16_NK	10.27	4.53	*		*		1.04	0.65		2.83	*
CD16neg_NK	*	*	*		*		0.62	0.79		13.73	*
CD56hi_NK	13.32	*	*		*		0.77	1.88		*	*
DC		11.33		*		*	0.63				*
cDC		12.52		*		*	0.87				*
pDC		*		*		*	1.57				*
transDC		*		*		*	*				*
MONO		10.37		2.90	*	*	0.74	*			*
cMONO		13.30		4.97	*	*	0.39	*			*
inMONO		*		5.09	*	*	1.50	*			*
ncMONO		*		4.89	*	*	4.25	*			*

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

MOMDSC		*		8.95	*	*	4.06	*			*
Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.											

Table 16: Intra-Run Precision (%CV) of Functional State Marker Frequencies for Donor 3 (HD6)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD38_ HLADR	CD74	CD8	CD86	CTLA4
BASOPHIL		0.73	*		15.29						
MAST_CELL		*	*		*						
NEUTROPHIL		0.01	2.69		*						
B_CELL		2.91			4.08			0.48		*	
B_NAIVE		3.35			*			0.37		*	
B_MEM		2.11			3.42			0.64		*	
PB		*			*			*		*	
T_CELL	0.22	2.13		0.66		0.16	8.15			15.26	6.53
GDT	9.91	1.64		0.83		0.54	*			*	*
NKT	*	1.61		2.40		3.46	*			*	*
DNT	5.15	0.77		1.38		6.45	*			*	*
DPT	9.93	5.17		*		*	*			*	*
TREG	2.00	2.02		3.47		1.39	*			*	8.27
CD4_nonTREG	0.29	2.11		0.61		0.48	*			*	10.57
CD4_TNAIVE		2.12		0.53		0.20	*			*	*
CD4_TCM	1.05	1.63		0.93		1.96	*			*	15.43
CD4_TEM	2.09	5.25		4.99		*	*			*	*
CD4_TEMRA	6.22	3.48		3.83		*	*			*	*
CD8_T	0.60	2.58		1.97		1.22	8.07			*	*
CD8_TNAIVE		2.33		1.64		1.22	*			*	*
CD8_TCM	3.93	0.71		1.60		3.53	*			*	*
CD8_TEM	*	*		*		*	*			*	*
CD8_TEMRA	*	6.70		7.12		2.92	*			*	*
NK		0.37		0.22		0.20			7.67	6.57	
CD16_NK		0.42		0.12		0.21			7.57	9.97	
CD16neg_NK		1.49		0.67		1.83			*	*	
CD56hi_NK		0.85		1.37		0.14			*	2.65	
DC		2.71						1.46		*	*
cDC		2.20						1.58		*	*
pDC		*						3.68		*	*
transDC		*						*		*	*
MONO		0.22	*					4.28		5.66	*
cMONO		0.02	*					3.88		5.57	*
inMONO		0.16	*					5.42		3.86	*
ncMONO		2.74	*					9.61		8.40	*

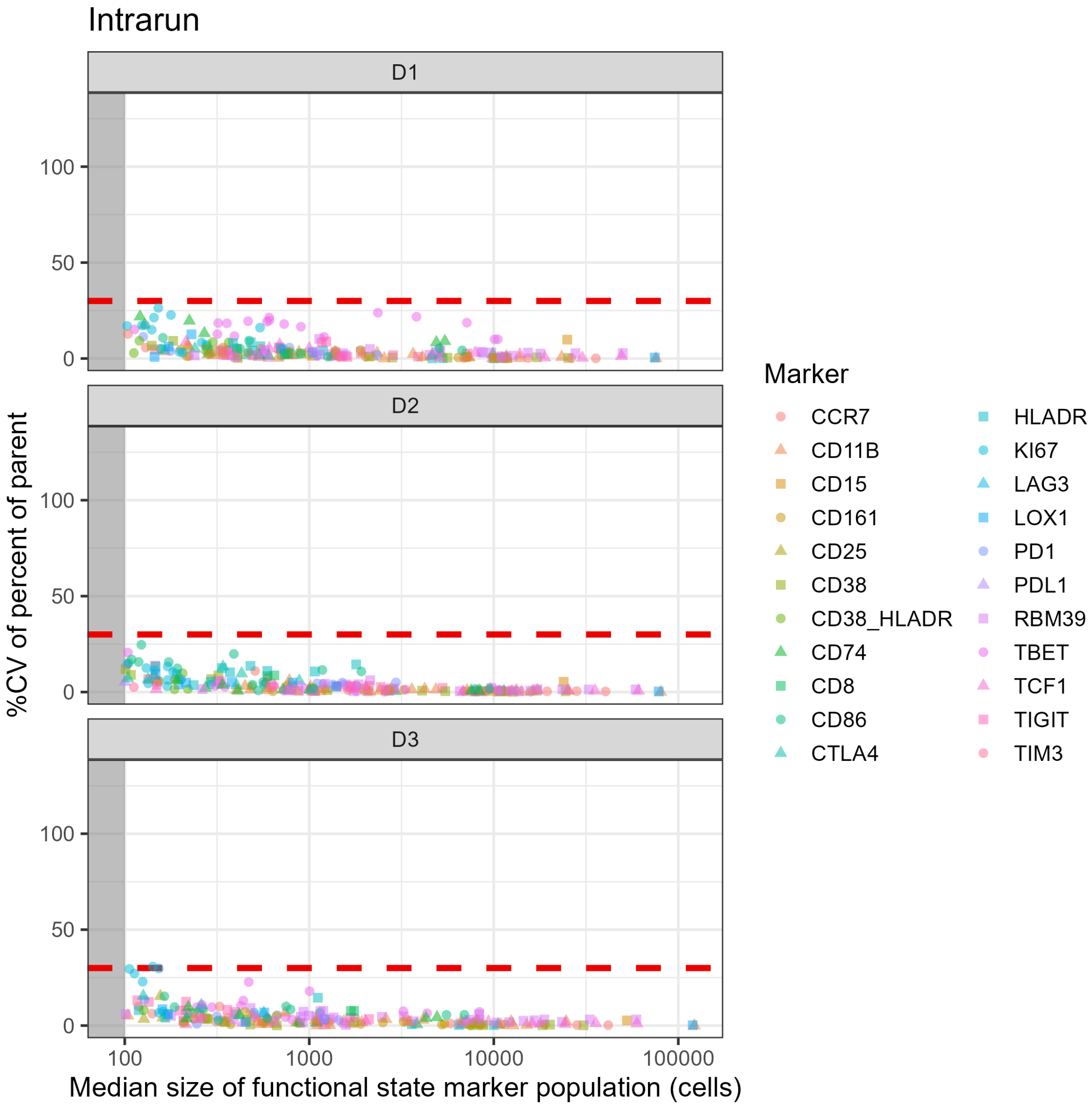
The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

MOMDSC		0.00	*					*		9.66	*
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
BASOPHIL	*	*		8.53		*	0.31				*
MAST_CELL	*	*		*		*	*				*
NEUTROPHIL	14.53	*		0.19		*	4.76				*
B_CELL	0.20	*	*			*	1.55	9.60			
B_NAIVE	0.30	*	*			*	1.20	*			
B_MEM	0.67	*	*			*	2.23	9.56			
PB	*	*	*			*	*	*			
T_CELL	5.81	6.77	*		2.95	*	3.19	7.12	1.06	2.79	*
GDT	*	*	*		*	*	4.57	3.88	2.17	12.35	*
NKT	*	*	*		*	*	4.23	4.43	1.39	13.18	*
DNT	*	*	*		*	*	3.21	10.40	1.92	12.45	*
DPT	*	*	*		*	*	2.52	*	0.60	*	*
TREG	*	*	*		*	*	1.54	*	0.10	2.28	*
CD4_nonTREG	8.05	9.26	*		5.39	*	3.18	17.91	1.17	2.28	*
CD4_TNAIVE	*	6.48	*		13.47	*	1.99	13.05	0.74	7.76	*
CD4_TCM	*	*	*		5.52	*	5.89	22.74	2.02	2.64	*
CD4_TEM	*	*	*		*	*	7.23	*	2.69	*	*
CD4_TEMRA	*	*	*		*	*	9.13	*	3.74	*	*
CD8_T	6.02	7.35	*		2.95	*	3.17	6.39	0.97	3.43	*
CD8_TNAIVE	*	6.67	*		4.86	*	2.77	7.49	0.93	4.05	*
CD8_TCM	*	*	*		0.89	*	7.47	10.83	2.44	7.94	*
CD8_TEM	*	*	*		*	*	5.97	*	*	*	*
CD8_TEMRA	*	*	*		*	*	5.19	3.16	1.40	3.73	*
NK	4.12	22.89	*		*		2.99	2.15		7.11	*
CD16_NK	*	*	*		*		3.09	2.38		8.20	*
CD16neg_NK	*	*	*		*		3.14	3.86		*	*
CD56hi_NK	*	*	*		*		2.32	4.19		*	*
DC		27.14		*		*	4.67				*
cDC		29.51		*		*	4.60				*
pDC		*		*		*	5.10				*
transDC		*		*		*	*				*
MONO		29.77		3.21	*	*	2.46	*			*
cMONO		30.76		6.85	*	*	1.84	*			*
inMONO		*		1.87	*	*	3.60	*			*
ncMONO		*		5.86	*	*	7.59	*			*
MOMDSC		*		*	*	*	1.65	*			*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Figure 7: Coefficient of Variation in Frequency (Percentage of Parent) and Median Number of Cells per Functional State Marker Positive Population for Intra-Run Precision Assessment



7. Intra-run Precision of Functional State Marker MCV

Coefficient of variation of functional state marker MCV between three replicates of the same run was calculated per subject for the intra-run precision analysis and is reported in Table 17-19 and Figure 8. All (545/545) measurable cell populations passed the acceptable threshold of ≤ 30% variation between replicates. **The median of intra-run %CV for all measurable populations across all three healthy subjects was 0.75%**, with individual median %CVs of 1.10%, 0.55%, and 0.83% for Donor 1 (HD4), Donor 2 (HD5), and Donor 3 (HD6), respectively.

Table 17: Intra-Run Precision (%CV) of Functional State Marker MCV for Donor 1 (HD4)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD74	CD8	CD86	CTLA4	
B_CELL		0.77			0.75		2.61		*		
B_MEM		0.53			1.15		2.42		*		
B_NAIVE		1.56			*		2.74		*		
BASOPHIL		0.66	*		0.34						
CD16_NK		0.32		0.13		0.10		0.61	0.09		
CD16neg_NK		2.32		3.61		3.26		*	*		
CD4_nonTREG	0.46	0.37		0.46		0.38			1.09	0.14	
CD4_TCM	0.29	0.40		0.65		0.42			*	0.29	
CD4_TEM	0.13	0.70		1.87		5.85			*	*	
CD4_TEMRA	*	*		*		*			*	*	
CD4_TNAIVE		0.38		*		0.38			*	*	
CD56hi_NK		0.71		3.24		0.33		*	0.90		
CD8_T	0.48	0.93		1.19		0.31			*	*	
CD8_TCM	0.66	1.23		2.73		1.32			*	*	
CD8_TEM	*	1.60		0.16		0.61			*	*	
CD8_TEMRA	*	2.19		*		2.65			*	*	
CD8_TNAIVE		1.25		*		0.31			*	*	
cDC		3.09					0.60		*	*	
cMONO		0.27	*				0.65		0.57	*	
DC		3.28					1.55		*	*	
DNT	0.36	1.25		0.14		2.57			*	*	
DPT	2.79	3.24		*		*			*	*	
GDT	1.62	0.33		1.43		2.98			*	*	
inMONO		3.15	*				1.30		0.56	*	
MAST_CELL		*	*		*						
MOMDSC		0.40	*				0.40		0.47	*	
MONO		0.31	*				0.63		0.39	*	
ncMONO		1.07	*				0.24		0.54	*	
NEUTROPHIL		2.91	0.35		*						
NK		0.39		0.15		0.11		0.55	0.31		
NKT	2.08	0.53		2.60		0.58			*	*	
PB		*			*		*		*		
pDC		*					*		*	*	
T_CELL	0.39	0.49		0.52		0.37			0.73	0.57	
transDC		*					*		*	*	
TREG	0.82	2.27		0.50		1.56			*	1.11	
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
B_CELL	0.17	*	*			*	2.91	1.19			

B_MEM	0.15	*	*			*	2.66	1.47			
B_NAIVE	0.25	*	*			*	3.27	*			
BASOPHIL	*	*		*		*	3.21				*
CD16_NK	0.63	1.57	*		*		2.43	5.44		0.61	*
CD16neg_NK	*	*	*		*		3.16	6.17		*	*
CD4_nonTREG	1.06	1.10	*		0.60	*	2.47	0.95	2.97	0.52	*
CD4_TCM	1.14	3.44	*		0.49	*	2.43	1.25	2.81	0.67	*
CD4_TEM	*	*	*		*	*	2.16	*	1.59	*	*
CD4_TEMRA	*	*	*		*	*	*	*	*	*	*
CD4_TNAIVE	*	*	*		*	*	2.59	*	3.18	*	*
CD56hi_NK	*	*	*		*		2.65	3.38		*	*
CD8_T	0.74	3.82	*		0.18	*	2.90	2.59	2.86	0.12	*
CD8_TCM	1.08	*	*		0.09	*	2.73	2.37	2.30	0.27	*
CD8_TEM	*	*	*		0.12	*	3.46	3.07	1.26	2.02	*
CD8_TEMRA	*	*	*		*	*	2.69	4.13	0.93	0.77	*
CD8_TNAIVE	*	*	*		*	*	3.09	2.63	3.63	1.22	*
cDC		0.63		*		*	1.95				*
cMONO		0.47		0.57	*	*	2.08	*			*
DC		0.83		*		*	2.12				*
DNT	*	*	*		0.72	*	2.78	2.59	3.01	0.84	*
DPT	*	*	*		*	*	2.76	*	2.84	*	*
GDT	*	*	*		*	*	2.96	2.92	2.12	3.38	*
inMONO		*		0.92	*	*	3.71	*			*
MAST_CELL	*	*		*		*	*				*
MOMDSC		*		0.70	*	*	2.91	*			*
MONO		0.26		1.56	*	*	2.10	*			*
ncMONO		*		1.08	*	*	2.21	*			*
NEUTROPHIL	2.87	0.34		3.07		*	1.86				*
NK	0.36	0.77	*		*		2.45	5.38		0.75	*
NKT	*	*	*		*	*	2.63	3.83	1.74	0.93	*
PB	*	*	*			*	*	*			
pDC		*		*		*	*				*
T_CELL	0.37	2.63	*		0.20	*	2.61	2.51	3.08	0.07	*
transDC		*		*		*	*				*
TREG	1.05	*	*		*	*	2.40	*	1.44	0.16	*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

Table 18: Intra-Run Precision (%CV) of Functional State Marker MCV for Donor 2 (HD5)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD74	CD8	CD86	CTLA4	
B_CELL		0.31			2.19		0.33		*		

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

B_MEM		0.65			1.78		0.17		*		
B_NAIVE		0.46			*		0.48		*		
BASOPHIL		0.16	*		1.50						
CD16_NK		0.16		0.59		0.22		0.09	1.07		
CD16neg_NK		0.86		0.60		0.07		*	1.68		
CD4_nonTREG	0.07	0.89		0.18		0.06			0.47	0.50	
CD4_TCM	0.08	0.96		0.34		0.46			1.12	0.50	
CD4_TEM	0.16	0.63		0.57		6.47			*	*	
CD4_TEMRA	*	*		*		*			*	*	
CD4_TNAIVE		1.19		*		0.36			*	*	
CD56hi_NK		0.70		1.93		0.32		*	0.21		
CD8_T	0.06	0.63		0.07		0.51			0.54	*	
CD8_TCM	0.13	0.74		0.24		1.03			*	*	
CD8_TEM	*	1.66		0.73		2.27			*	*	
CD8_TEMRA	*	*		*		*			*	*	
CD8_TNAIVE		1.58		*		0.36			*	*	
cDC		2.31					0.02		0.64	*	
cMONO		0.07	*				0.06		0.36	*	
DC		2.84					0.71		0.55	*	
DNT	1.21	0.48		0.31		0.92			*	*	
DPT	*	*		*		*			*	*	
GDT	0.74	0.88		0.68		2.40			*	*	
inMONO		1.82	*				0.32		0.94	*	
MAST_CELL		*	*		*						
MOMDSC		0.62	*				0.12		1.47	*	
MONO		0.05	*				0.12		0.39	0.44	
ncMONO		0.88	*				0.93		0.77	0.35	
NEUTROPHIL		1.75	0.37		*						
NK		0.19		0.39		0.23		0.29	0.81		
NKT	*	1.39		0.44		*			*	*	
PB		*			*		*		*		
pDC		5.62					2.52		*	*	
T_CELL	0.08	0.56		0.44		0.12			0.28	1.09	
transDC		*					*		*	*	
TREG	1.58	1.84		3.68		1.36			*	1.48	
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
B_CELL	0.52	*	*			*	0.40	3.19			
B_MEM	0.89	*	*			*	0.62	*			
B_NAIVE	0.52	*	*			*	0.29	*			
BASOPHIL	*	*		2.29		*	0.31				*
CD16_NK	0.29	3.78	*		*		0.45	0.54		0.08	*

CD16neg_NK	*	*	*		*		0.28	0.41		1.29	*
CD4_nonTREG	0.65	1.78	*		0.23	*	0.37	0.45	0.14	0.47	*
CD4_TCM	0.36	3.38	*		0.20	*	0.34	0.45	0.09	0.56	*
CD4_TEM	*	*	*		*	*	0.88	1.24	0.52	*	*
CD4_TEMRA	*	*	*		*	*	*	*	*	*	*
CD4_TNAIVE	*	*	*		*	*	0.46	*	0.22	*	*
CD56hi_NK	0.09	*	*		*		0.64	1.90		*	*
CD8_T	1.63	0.55	*		0.55	*	0.24	0.33	0.39	0.63	*
CD8_TCM	1.19	*	*		0.44	*	0.30	0.18	0.32	0.66	*
CD8_TEM	*	*	*		1.63	*	0.25	0.74	0.38	0.82	*
CD8_TEMRA	*	*	*		*	*	*	*	*	*	*
CD8_TNAIVE	*	*	*		*	*	0.31	2.81	0.41	1.99	*
cDC		3.14		*		*	0.56				*
cMONO		1.18		0.21	*	*	0.64	*			*
DC		2.36		*		*	0.92				*
DNT	*	*	*		2.38	*	0.28	0.66	0.38	0.85	*
DPT	*	*	*		*	*	0.73	*	1.00	*	*
GDT	*	*	*		*	*	0.22	1.03	0.19	*	*
inMONO		*		0.97	*	*	1.06	*			*
MAST_CELL	*	*		*		*	*				*
MOMDSC		*		0.60	*	*	1.22	*			*
MONO		0.91		0.66	*	*	0.63	*			*
ncMONO		*		0.74	*	*	1.48	*			*
NEUTROPHIL	0.31	*		2.05		*	0.36				*
NK	0.38	2.97	*		*		0.44	0.39		0.30	0.67
NKT	*	*	*		*	*	0.23	1.27	0.58	*	*
PB	*	*	*			*	*	*			
pDC		*		*		*	1.80				*
T_CELL	1.02	0.75	*		0.42	0.23	0.32	0.42	0.18	0.47	*
transDC		*		*		*	*				*
TREG	1.85	*	*		*	*	0.38	*	0.57	0.88	*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

Table 19: Intra-Run Precision (%CV) of Functional State Marker MCV for Donor 3 (HD6)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD74	CD8	CD86	CTLA4	
B_CELL		0.54			1.08		1.51		*		
B_MEM		1.21			0.24		0.86		*		
B_NAIVE		1.41			*		1.87		*		
BASOPHIL		0.45	*		2.11						
CD16_NK		0.74		0.24		0.22		0.69	0.11		

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

CD16neg_NK		1.61		0.36		0.66		*	*		
CD4_nonTREG	0.06	0.23		0.30		0.14			*	0.63	
CD4_TCM	0.25	0.31		0.43		0.85			*	0.71	
CD4_TEM	0.84	1.05		0.57		*			*	*	
CD4_TEMRA	0.73	1.62		2.70		*			*	*	
CD4_TNAIVE		0.19		0.85		0.13			*	*	
CD56hi_NK		1.01		1.75		0.47		*	0.44		
CD8_T	0.27	0.87		0.73		0.36			*	*	
CD8_TCM	0.87	0.52		6.39		1.77			*	*	
CD8_TEM	*	*		*		*			*	*	
CD8_TEMRA	*	1.35		1.63		1.07			*	*	
CD8_TNAIVE		0.85		0.07		0.35			*	*	
cDC		2.46					0.33		*	*	
cMONO		0.16	*				0.26		0.90	*	
DC		1.84					0.21		*	*	
DNT	2.94	2.08		0.52		4.23			*	*	
DPT	0.91	1.59		*		*			*	*	
GDT	0.74	1.01		0.50		2.36			*	*	
inMONO		1.43	*				0.17		1.76	*	
MAST_CELL		*	*		*						
MOMDSC		0.89	*				*		2.17	*	
MONO		0.27	*				0.18		0.89	*	
ncMONO		1.98	*				0.75		0.79	*	
NEUTROPHIL		0.86	0.20		*						
NK		0.81		0.16		0.20		0.55	0.17		
NKT	*	0.50		0.72		1.94			*	*	
PB		*			*		*		*		
pDC		*					0.37		*	*	
T_CELL	0.11	0.30		0.28		0.22			1.14	0.49	
transDC		*					*		*	*	
TREG	0.76	3.01		2.84		1.35			*	2.17	
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
B_CELL	0.42	*	*			*	0.60	1.66			
B_MEM	0.63	*	*			*	0.41	2.40			
B_NAIVE	0.45	*	*			*	0.71	*			
BASOPHIL	*	*		3.44		*	0.59				*
CD16_NK	*	*	*		*		0.49	1.72		1.26	*
CD16neg_NK	*	*	*		*		0.28	2.53		*	*
CD4_nonTREG	1.16	1.28	*		0.94	*	0.71	1.05	0.58	1.32	*
CD4_TCM	*	*	*		1.47	*	0.88	1.31	0.28	1.67	*
CD4_TEM	*	*	*		*	*	0.68	*	0.67	*	*

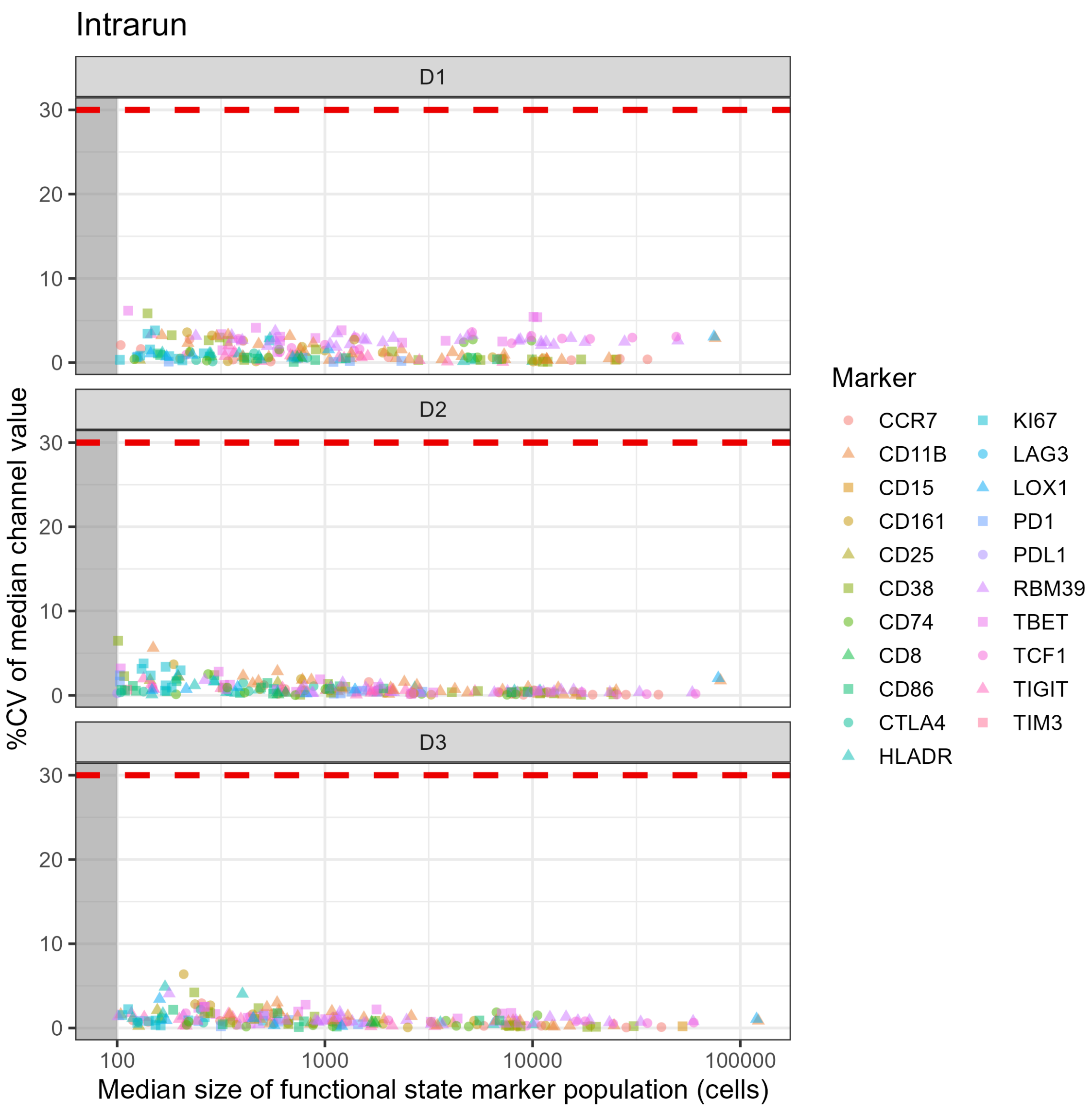
The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

CD4_TEMRA	*	*	*		*	*	0.43	*	0.99	*	*
CD4_TNAIVE	*	0.91	*		0.85	*	0.67	0.53	0.50	1.06	*
CD56hi_NK	*	*	*		*		0.86	1.68		*	*
CD8_T	4.88	0.30	*		0.75	*	0.89	1.13	0.80	0.28	*
CD8_TCM	*	*	*		0.82	*	1.25	2.51	0.46	0.57	*
CD8_TEM	*	*	*		*	*	1.37	*	*	*	*
CD8_TEMRA	*	*	*		*	*	0.80	2.78	1.39	0.83	*
CD8_TNAIVE	*	0.37	*		0.21	*	0.87	0.74	0.78	0.20	*
cDC		1.54		*		*	0.91				*
cMONO		0.81		0.32	*	*	1.28	*			*
DC		2.24		*		*	1.14				*
DNT	*	*	*		*	*	0.64	1.22	1.08	0.26	*
DPT	*	*	*		*	*	1.44	*	1.75	*	*
GDT	*	*	*		*	*	0.94	2.20	0.92	1.29	*
inMONO		*		1.16	*	*	1.86	*			*
MAST_CELL	*	*		*		*	*				*
MOMDSC		*		*	*	*	0.79	*			*
MONO		0.90		0.22	*	*	1.32	*			*
ncMONO		*		0.92	*	*	1.41	*			*
NEUTROPHIL	0.68	*		1.07		*	0.47				*
NK	1.70	0.61	*		*		0.50	1.79		1.07	*
NKT	*	*	*		*	*	0.78	1.95	1.34	1.74	*
PB	*	*	*			*	*	*			
pDC		*		*		*	4.08				*
T_CELL	4.05	0.86	*		0.45	*	0.79	0.86	0.64	0.21	*
transDC		*		*		*	*				*
TREG	*	*	*		*	*	0.54	*	0.43	0.29	*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Figure 8: Coefficient of Variation in Functional State Marker MCV and Median Number of Cells per Functional State Marker Positive Population for Intra-Run Precision Assessment



8. Inter-run Precision of Functional State Marker Frequency

Coefficient of variation of functional state marker frequency between three replicates of the three runs was calculated per subject for the inter-run precision analysis and is reported in Table 20-22 and Figure 9. 79.18% (483/610) measurable cell populations passed the acceptable threshold of ≤ 30% variation between replicates. **The median of inter-run %CV for all measurable populations across all three healthy subjects was 7.86%**, with individual median %CVs of 7.24%, 7.73%, and 9.02% for Donor 1 (HD4), Donor 2 (HD5), and Donor 3

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

(HD6), respectively.

Table 20: Inter-Run Precision (%CV) of Functional State Marker Frequencies for Donor 1 (HD4)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD38_ HLADR	CD74	CD8	CD86	CTLA4
BASOPHIL		5.43	*		50.81						
MAST_CELL		*	*		*						
NEUTROPHIL		0.25	51.97		45.74						
B_CELL		31.75			70.73			0.19		*	
B_MEM		30.27			71.37			0.24		*	
B_NAIVE		33.99			*			0.21		*	
PB		*			*			*		*	
T_CELL	5.77	27.87		2.11		2.52	5.42			99.43	12.28
GDT	36.66	10.32		0.55		9.80	*			*	*
NKT	41.28	6.75		6.22		7.25	*			*	*
DNT	6.77	21.11		2.05		2.37	*			*	*
DPT	14.83	32.34		*		11.58	*			*	*
TREG	11.03	38.61		0.14		5.30	4.66			*	11.59
CD4_nonTREG	5.34	36.53		1.77		1.55	6.03			98.91	16.53
CD4_TNAIVE		38.27		*		0.98	*			93.14	*
CD4_TCM	9.24	36.39		1.73		4.68	6.67			105.16	13.98
CD4_TEM	24.36	32.45		3.17		6.03	*			*	*
CD4_TEMRA	*	*		*		*	*			*	*
CD8_T	5.81	24.93		2.09		5.12	7.01			100.85	*
CD8_TNAIVE		33.00		6.76		5.63	*			*	*
CD8_TCM	20.36	25.26		1.33		5.13	12.16			*	*
CD8_TEM	*	14.94		6.42		5.62	*			*	*
CD8_TEMRA	*	12.53		4.92		4.60	*			*	*
NK		2.27		0.95		0.22			34.65	56.37	
CD16_NK		1.90		0.90		0.24			37.91	62.21	
CD16neg_NK		13.67		4.30		2.43			*	*	
CD56hi_NK		3.68		0.84		1.19			*	25.10	
DC		28.21						1.52		51.65	*
cDC		28.61						1.20		51.30	*
pDC		*						5.92		*	*
transDC		*						*		*	*
MONO		1.09	*					4.91		40.92	*
cMONO		0.05	*					3.78		51.01	*
inMONO		0.42	*					9.36		32.31	*
ncMONO		9.80	*					14.22		8.39	*
MOMDSC		0.01	*					10.96		93.40	*
Functional State	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3

Marker											
BASOPHIL	*	*		*		*	12.82				*
MAST_CELL	*	*		*		*	*				*
NEUTROPHIL	4.83	*		5.37		*	67.65				*
B_CELL	0.89	*	*			*	0.86	9.75			
B_MEM	0.91	*	*			*	0.86	9.15			
B_NAIVE	0.94	*	*			*	0.90	*			
PB	2.56	*	*			*	0.36	*			
T_CELL	10.84	22.08	47.71		21.88	*	2.28	10.58	0.56	7.49	29.86
GDT	*	*	*		*	*	1.57	3.91	1.75	5.34	*
NKT	*	*	*		*	*	2.53	2.60	6.66	4.06	*
DNT	*	*	*		27.60	*	0.51	9.95	0.80	4.78	*
DPT	*	*	*		*	*	4.76	*	1.92	*	*
TREG	9.89	23.13	*		*	*	1.88	*	1.98	8.42	*
CD4_nonTREG	14.91	20.13	*		22.22	*	2.79	34.62	0.76	10.34	*
CD4_TNAIVE	*	*	*		*	*	2.63	*	0.91	*	*
CD4_TCM	18.93	17.06	*		20.31	*	2.76	32.38	0.71	10.49	*
CD4_TEM	17.29	*	*		*	*	4.85	*	2.55	*	*
CD4_TEMRA	*	*	*		*	*	*	*	*	*	*
CD8_T	11.10	24.50	*		21.43	*	1.97	10.08	0.43	6.90	*
CD8_TNAIVE	*	*	*		*	*	2.29	15.23	0.92	7.24	*
CD8_TCM	14.54	24.14	*		19.80	*	1.79	12.92	0.62	7.55	*
CD8_TEM	*	*	*		25.67	*	2.60	7.28	3.52	10.67	*
CD8_TEMRA	*	*	*		*	*	1.42	3.60	5.63	3.05	*
NK	25.32	38.03	*		*		1.00	2.40		15.07	65.99
CD16_NK	34.42	40.09	*		*		0.93	2.26		15.64	64.58
CD16neg_NK	*	*	*		*		1.79	8.74		14.76	*
CD56hi_NK	*	*	*		*		2.48	5.57		2.61	*
DC		42.29		*		*	2.41				43.65
cDC		40.65		*		*	2.31				45.45
pDC		*		*		*	10.39				*
transDC		*		*		*	*				*
MONO		38.06		13.38	*	*	0.45	*			*
cMONO		39.14		25.44	*	*	0.38	*			*
inMONO		*		20.48	*	*	5.28	*			*
ncMONO		*		7.99	*	*	2.43	*			*
MOMDSC		*		9.73	*	*	0.50	*			*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

Table 21: Inter-Run Precision (%CV) of Functional State Marker for Donor 2 (HD5)

Functional State	CCR7	CD11B	CD15	CD161	CD25	CD38	CD38_	CD74	CD8	CD86	CTLA4

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Marker							HLADR				
BASOPHIL		2.42	*		35.23						
MAST_CELL		*	*		*						
NEUTROPHIL		0.35	44.46		*						
B_CELL		29.84			60.32			0.11		*	
B_MEM		32.98			57.22			0.19		*	
B_NAIVE		28.77			*			0.10		*	
PB		*			*			*		*	
T_CELL	6.14	28.72		2.33		1.99	20.74			105.87	13.87
GDT	21.18	11.61		1.52		7.35	*			*	*
NKT	33.88	22.33		3.58		36.85	*			*	*
DNT	14.27	26.86		0.42		9.73	*			*	*
DPT	13.20	*		*		*	*			*	*
TREG	10.05	34.97		7.37		4.61	23.12			*	4.43
CD4_nonTREG	5.72	33.09		3.51		0.83	26.76			110.50	17.89
CD4_TNAIVE		37.02		*		0.50	*			*	*
CD4_TCM	9.02	32.86		2.77		4.08	20.06			113.03	19.59
CD4_TEM	19.18	27.09		3.32		5.23	*			*	*
CD4_TEMRA	*	*		*		*	*			*	*
CD8_T	5.33	29.15		2.23		4.29	17.28			102.29	*
CD8_TNAIVE		34.01		*		3.52	*			*	*
CD8_TCM	20.52	28.25		2.00		7.71	17.54			98.92	*
CD8_TEM	*	20.00		4.04		5.59	*			*	*
CD8_TEMRA	*	*		*		*	*			*	*
NK		3.63		0.83		0.16			31.52	51.29	
CD16_NK		3.04		0.89		0.31			34.17	64.47	
CD16neg_NK		6.29		1.23		2.16			*	51.67	
CD56hi_NK		4.11		1.33		0.21			*	18.87	
DC		22.94						4.00		39.47	*
cDC		20.61						2.93		38.33	*
pDC		*						7.61		*	*
transDC		*						*		*	*
MONO		0.66	*					6.89		28.07	*
cMONO		0.07	*					6.49		30.29	*
inMONO		0.76	*					7.21		28.34	*
ncMONO		9.40	*					13.20		12.31	*
MOMDSC		0.00	*					27.26		68.52	*
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
BASOPHIL	*	*		2.57		*	7.24				*
MAST_CELL	*	*		*		*	*				*
NEUTROPHIL	62.72	*		5.09		*	45.27				*

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

B_CELL	1.94	*	*			*	0.70	7.68			
B_MEM	2.75	*	*			*	0.41	*			
B_NAIVE	1.67	*	*			*	0.81	*			
PB	*	*	*			*	*	*			
T_CELL	29.51	12.43	*		21.09	*	1.12	9.62	0.12	9.15	*
GDT	*	*	*		16.09	*	1.09	2.04	2.39	9.94	*
NKT	*	*	*		*	*	3.92	7.73	1.41	6.67	*
DNT	*	*	*		30.27	*	0.57	14.79	0.77	5.10	*
DPT	*	*	*		*	*	4.54	*	4.65	*	*
TREG	23.36	6.06	*		*	*	2.34	*	1.62	6.44	*
CD4_nonTREG	35.48	11.31	*		21.20	*	1.77	22.32	0.44	11.29	*
CD4_TNAIVE	*	*	*		*	*	1.83	*	0.40	*	*
CD4_TCM	31.80	11.54	*		20.19	*	1.92	24.68	0.53	11.38	*
CD4_TEM	*	*	*		22.42	*	1.59	3.58	0.56	*	*
CD4_TEMRA	*	*	*		*	*	*	*	*	*	*
CD8_T	24.84	15.87	*		21.33	*	0.60	7.63	0.18	9.19	*
CD8_TNAIVE	*	*	*		*	*	0.94	11.90	0.43	9.23	*
CD8_TCM	24.10	14.22	*		19.83	*	0.99	8.71	0.48	9.57	*
CD8_TEM	*	*	*		29.27	*	0.94	2.60	1.29	6.82	*
CD8_TEMRA	*	*	*		*	*	4.02	*	4.45	*	*
NK	35.25	17.34	*		*		1.17	1.16		15.35	67.60
CD16_NK	44.50	18.78	*		*		1.19	1.04		15.96	*
CD16neg_NK	*	*	*		*		1.70	2.60		15.36	*
CD56hi_NK	33.08	*	*		*		0.98	2.99		9.06	*
DC		44.90		*		*	4.69				53.16
cDC		36.26		*		*	3.09				52.88
pDC		*		*		*	9.95				*
transDC		*		*		*	*				*
MONO		45.12		11.13	*	*	1.35	*			*
cMONO		*		24.64	*	*	0.91	*			*
inMONO		*		11.26	*	*	2.37	*			*
ncMONO		*		22.82	*	*	5.75	*			*
MOMDSC		*		*	*	*	3.86	*			*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

Table 22: Inter-Run Precision (%CV) of Functional State Marker for Donor 3 (HD6)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD38_HLADR	CD74	CD8	CD86	CTLA4
BASOPHIL		2.42	*		103.70						
MAST_CELL		*	*		*						
NEUTROPHIL		0.13	35.34		53.26						

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

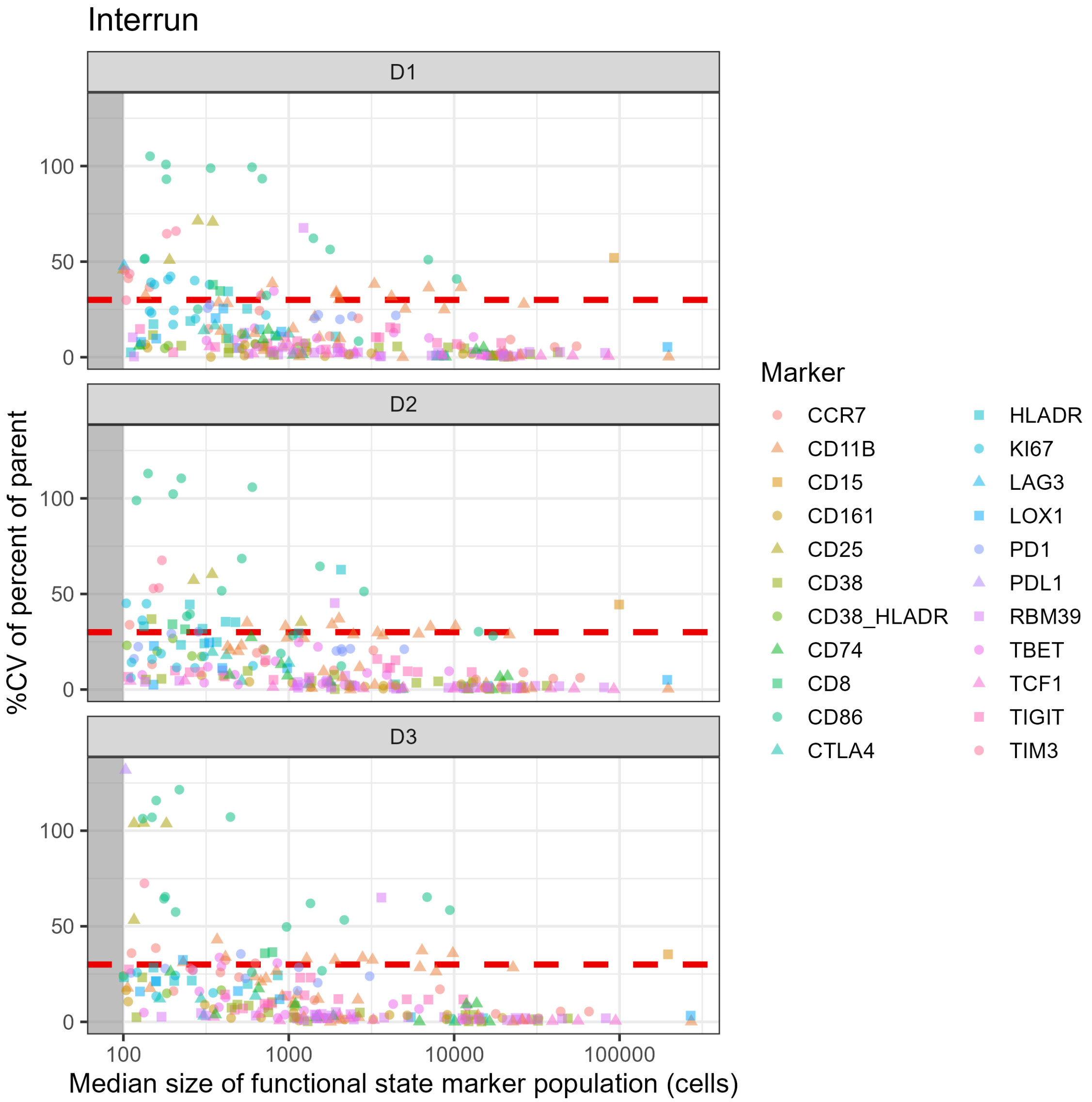
B_CELL		32.39			103.96			0.10		*	
B_MEM		33.01			103.78			0.14		*	
B_NAIVE		32.34			*			0.05		*	
PB		*			*			*		*	
T_CELL	5.40	28.43		1.89		1.79	16.58			107.17	13.53
GDT	32.20	11.55		1.30		10.37	*			*	*
NKT	35.95	14.12		1.22		8.51	*			*	*
DNT	23.37	26.54		2.23		7.40	*			*	*
DPT	16.12	17.75		16.49		23.26	*			*	*
TREG	11.37	42.99		9.00		8.44	*			*	3.10
CD4_nonTREG	5.45	35.86		2.11		0.56	24.00			115.80	12.02
CD4_TNAIVE		37.29		3.71		1.35	*			*	*
CD4_TCM	17.06	33.55		1.29		4.89	*			*	12.10
CD4_TEM	25.87	33.91		4.76		*	*			*	*
CD4_TEMRA	38.59	31.11		2.03		*	*			*	*
CD8_T	4.17	26.29		3.41		3.67	14.93			107.01	*
CD8_TNAIVE		28.49		3.61		3.59	*			106.32	*
CD8_TCM	30.64	23.68		6.68		9.05	*			*	*
CD8_TEM	*	17.59		10.60		2.44	*			*	*
CD8_TEMRA	*	13.39		2.26		5.00	*			*	*
NK		3.26		0.82		0.21			36.45	53.32	
CD16_NK		2.57		0.66		0.26			35.88	61.97	
CD16neg_NK		9.66		0.68		2.18			*	57.47	
CD56hi_NK		2.38		2.40		0.30			*	25.97	
DC		22.61						2.79		65.46	*
cDC		21.08						2.42		64.38	*
pDC		*						3.83		*	*
transDC		*						*		*	*
MONO		0.81	*					9.56		58.46	*
cMONO		0.05	*					9.11		65.26	*
inMONO		0.95	*					9.17		49.67	*
ncMONO		11.91	*					17.42		26.72	*
MOMDSC		0.00	*					26.17		121.47	*
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
BASOPHIL	*	*		15.90		*	5.10				*
MAST_CELL	*	*		*		*	*				*
NEUTROPHIL	24.32	*		3.25		131.77	65.03				*
B_CELL	1.93	*	*			*	2.43	12.77			
B_MEM	2.50	*	*			*	2.42	15.95			
B_NAIVE	1.57	*	*			*	2.53	*			
PB	*	*	*			*	*	*			

T_CELL	19.81	15.17	*		23.88	*	1.88	8.26	0.50	11.73	*
GDT	*	*	*		25.55	*	2.24	2.74	0.90	6.32	*
NKT	*	*	*		*	*	1.23	5.85	3.37	14.29	*
DNT	*	*	*		27.91	*	4.01	7.21	1.90	8.66	*
DPT	*	*	*		*	*	4.63	*	2.63	*	*
TREG	23.34	*	*		*	*	4.14	*	3.07	7.04	*
CD4_nonTREG	21.14	13.38	*		20.47	*	1.69	30.77	0.53	11.93	*
CD4_TNAIVE	*	*	*		27.04	*	1.36	33.66	0.36	9.54	*
CD4_TCM	*	*	*		16.32	*	1.96	28.98	1.18	13.90	*
CD4_TEM	*	*	*		*	*	2.44	*	1.27	*	*
CD4_TEMRA	*	*	*		*	*	3.30	*	0.71	*	*
CD8_T	21.59	*	*		28.57	*	2.01	6.74	0.65	11.67	*
CD8_TNAIVE	28.41	*	*		35.53	*	2.04	9.30	0.72	11.80	*
CD8_TCM	*	*	*		22.77	*	2.00	7.68	1.73	13.63	*
CD8_TEM	*	*	*		*	*	2.67	4.84	*	*	*
CD8_TEMRA	*	*	*		*	*	1.87	1.69	4.16	9.72	*
NK	21.41	24.26	*		*		1.24	1.22		23.16	72.46
CD16_NK	*	25.86	*		*		1.34	1.01		23.12	*
CD16neg_NK	*	*	*		*		1.73	2.06		27.43	*
CD56hi_NK	*	*	*		*		0.86	3.79		*	*
DC		*		*		*	3.36				27.00
cDC		*		*		*	3.49				28.09
pDC		*		*		*	3.23				*
transDC		*		*		*	*				*
MONO		*		11.85	*	*	1.08	*			*
cMONO		*		32.42	*	*	0.94	*			*
inMONO		*		16.13	*	*	1.87	*			*
ncMONO		*		21.36	*	*	1.98	*			*
MOMDSC		*		*	*	*	3.86	*			*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Figure 9: Coefficient of Variation in Frequency (Percentage of Parent) and Median Number of Cells per Functional State Marker Positive Population for Inter-Run Precision Assessment



9. Inter-run Precision of Functional State Marker MCV

Coefficient of variation of functional state marker MCV between three replicates of the three runs was calculated per subject for the inter-run precision analysis and is reported in Table 23-25 and Figure 10. All (595/595) measurable cell populations passed the acceptable threshold of ≤ 30% variation between replicates. **The median of inter-run %CV for all measurable populations across all three healthy subjects was 2.17%**, with individual median %CVs of 2.21%, 2.12%, and 2.22% for Donor 1 (HD4), Donor 2 (HD5), and Donor 3

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

(HD6), respectively.

Table 23: Inter-Run Precision (%CV) of Functional State Marker MCV for Donor 1 (HD4)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD74	CD8	CD86	CTLA4	
B_CELL		4.40			2.94		0.55		*		
B_MEM		4.92			2.79		0.70		*		
B_NAIVE		3.80			*		0.62		*		
BASOPHIL		9.79	*		4.50						
CD16_NK		9.33		0.26		0.34		5.03	1.69		
CD16neg_NK		7.51		2.13		1.12		*	*		
CD4_nonTREG	1.21	4.05		0.53		0.22			0.62	1.91	
CD4_TCM	1.50	4.19		0.68		0.26			0.78	1.46	
CD4_TEM	2.17	4.32		0.63		2.81			*	*	
CD4_TEMRA	*	*		*		*			*	*	
CD4_TNAIVE		3.67		*		0.01			0.55	*	
CD56hi_NK		9.76		1.54		0.38		*	1.97		
CD8_T	1.18	5.94		2.91		0.26			0.78	*	
CD8_TCM	2.40	5.81		3.68		0.44			*	*	
CD8_TEM	*	9.22		2.95		1.60			*	*	
CD8_TEMRA	*	10.49		2.84		1.72			*	*	
CD8_TNAIVE		4.06		6.28		0.28			*	*	
cDC		9.11					1.13		2.37	*	
cMONO		5.52	*				0.90		5.95	*	
DC		7.81					1.35		2.31	*	
DNT	2.26	6.73		0.44		1.47			*	*	
DPT	2.57	10.39		*		3.07			*	*	
GDT	2.05	11.03		0.38		0.77			*	*	
inMONO		10.93	*				1.62		9.01	*	
MAST_CELL		*	*		*						
MOMDSC		3.14	*				0.35		5.10	*	
MONO		5.63	*				0.91		5.06	*	
ncMONO		7.29	*				1.36		4.67	*	
NEUTROPHIL		9.07	5.30		1.40						
NK		9.30		0.27		0.30		5.32	1.52		
NKT	6.39	9.46		1.88		1.60			*	*	
PB		*			*		*		*		
pDC		*					4.92		*	*	
T_CELL	1.25	4.87		0.53		0.17			0.71	0.77	
transDC		*					*		*	*	
TREG	2.31	6.14		1.48		2.49			*	1.28	
Functional State	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Marker											
B_CELL	4.30	*	*			*	2.49	2.68			
B_MEM	4.04	*	*			*	3.09	2.48			
B_NAIVE	4.46	*	*			*	2.12	*			
BASOPHIL	*	*		*		*	1.26				*
CD16_NK	2.78	4.31	*		*		0.93	2.35		1.02	1.16
CD16neg_NK	*	*	*		*		2.48	3.28		2.00	*
CD4_nonTREG	1.90	1.55	*		0.23	*	2.40	0.13	2.64	1.46	*
CD4_TCM	2.86	1.03	*		0.26	*	2.61	0.33	2.51	1.49	*
CD4_TEM	0.77	*	*		*	*	3.00	*	2.87	*	*
CD4_TEMRA	*	*	*		*	*	*	*	*	*	*
CD4_TNAIVE	*	*	*		*	*	2.15	*	2.86	*	*
CD56hi_NK	*	*	*		*		0.13	3.86		2.95	*
CD8_T	3.08	2.04	*		1.11	*	2.53	1.01	2.13	2.16	*
CD8_TCM	2.51	1.97	*		1.08	*	2.50	1.08	1.80	2.02	*
CD8_TEM	*	*	*		1.31	*	2.60	2.36	2.52	1.57	*
CD8_TEMRA	*	*	*		*	*	2.21	1.78	1.68	3.89	*
CD8_TNAIVE	*	*	*		*	*	2.58	1.92	2.56	3.34	*
cDC		1.35		*		*	2.58				3.81
cMONO		1.26		1.08	*	*	1.76	*			*
DC		1.54		*		*	2.51				3.47
DNT	*	*	*		1.09	*	2.47	1.44	2.34	2.58	*
DPT	*	*	*		*	*	3.09	*	2.72	*	*
GDT	*	*	*		*	*	3.29	2.05	1.59	2.27	*
inMONO		*		2.47	*	*	4.28	*			*
MAST_CELL	*	*		*		*	*				*
MOMDSC		*		0.92	*	*	0.67	*			*
MONO		1.81		1.94	*	*	1.77	*			*
ncMONO		*		3.17	*	*	2.05	*			*
NEUTROPHIL	7.89	*		11.10		*	4.03				*
NK	2.32	3.50	*		*		0.93	2.36		0.99	1.01
NKT	*	*	*		*	*	2.92	2.03	1.06	2.93	*
PB	4.18	*	*			*	1.70	*			
pDC		*		*		*	5.74				*
T_CELL	1.82	1.81	1.22		0.67	*	2.50	0.91	2.56	2.01	2.54
transDC		*		*		*	*				*
TREG	1.29	5.41	*		*	*	2.17	*	2.26	1.07	*
Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.											

Table 24: Inter-Run Precision (%CV) of Functional State Marker MCV for Donor 2 (HD5)

Functional State	CCR7	CD11B	CD15	CD161	CD25	CD38	CD74	CD8	CD86	CTLA4	

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

Marker											
B_CELL		3.38			2.00		1.05		*		
B_MEM		3.05			2.05		1.19		*		
B_NAIVE		3.63			*		1.04		*		
BASOPHIL		10.36	*		5.29						
CD16_NK		8.68		0.43		0.51		8.61	1.81		
CD16neg_NK		8.06		1.63		1.15		*	2.75		
CD4_nonTREG	1.81	3.29		0.14		0.50			1.24	1.58	
CD4_TCM	2.28	3.28		0.12		1.10			1.29	1.46	
CD4_TEM	1.61	5.34		1.40		0.72			*	*	
CD4_TEMRA	*	*		*		*			*	*	
CD4_TNAIVE		3.09		*		0.56			*	*	
CD56hi_NK		10.13		0.70		0.65		*	3.81		
CD8_T	1.41	3.78		0.56		0.36			1.05	*	
CD8_TCM	2.38	3.70		0.46		2.12			1.66	*	
CD8_TEM	*	8.93		0.69		2.34			*	*	
CD8_TEMRA	*	*		*		*			*	*	
CD8_TNAIVE		3.30		*		0.60			*	*	
cDC		3.82					0.95		0.99	*	
cMONO		5.66	*				1.09		5.43	*	
DC		4.00					1.41		0.73	*	
DNT	1.48	3.88		0.20		3.35			*	*	
DPT	7.71	*		*		*			*	*	
GDT	2.33	7.94		0.28		0.58			*	*	
inMONO		8.57	*				1.81		5.96	*	
MAST_CELL		*	*		*						
MOMDSC		3.43	*				0.75		5.38	*	
MONO		5.63	*				1.09		5.18	*	
ncMONO		6.70	*				1.64		3.81	*	
NEUTROPHIL		6.52	4.02		*						
NK		8.94		0.45		0.48		9.11	1.67		
NKT	5.45	7.19		0.35		5.27			*	*	
PB		*			*		*		*		
pDC		*					2.01		*	*	
T_CELL	1.79	3.83		0.43		0.56			1.14	1.34	
transDC		*					*		*	*	
TREG	1.65	4.08		3.20		1.42			*	0.81	
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
B_CELL	4.01	*	*			*	2.73	11.77			
B_MEM	3.74	*	*			*	3.35	*			
B_NAIVE	3.99	*	*			*	2.61	*			

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

BASOPHIL	*	*		4.68		*	1.33				*
CD16_NK	7.46	4.23	*		*		0.99	1.74		0.89	*
CD16neg_NK	*	*	*		*		1.68	1.17		2.36	*
CD4_nonTREG	9.05	6.07	*		0.91	*	2.22	0.72	2.79	0.98	*
CD4_TCM	10.50	4.77	*		0.72	*	2.34	0.52	2.55	0.96	*
CD4_TEM	*	*	*		1.48	*	1.91	0.41	1.79	*	*
CD4_TEMRA	*	*	*		*	*	*	*	*	*	*
CD4_TNAIVE	*	*	*		*	*	1.98	*	3.04	*	*
CD56hi_NK	5.53	*	*		*		1.79	1.37		5.43	*
CD8_T	8.32	2.31	*		0.79	*	2.91	0.17	2.71	1.69	*
CD8_TCM	7.34	5.64	*		1.03	*	2.79	0.42	2.20	1.54	*
CD8_TEM	*	*	*		0.61	*	3.38	1.10	4.12	2.77	*
CD8_TEMRA	*	*	*		*	*	1.97	*	6.80	*	*
CD8_TNAIVE	*	*	*		*	*	3.02	1.67	2.97	3.58	*
cDC		2.38		*		*	1.25				2.69
cMONO		*		1.30	*	*	1.58	*			*
DC		1.98		*		*	0.83				2.70
DNT	*	*	*		1.62	*	3.17	0.86	2.30	1.53	*
DPT	*	*	*		*	*	1.72	*	4.91	*	*
GDT	*	*	*		1.82	*	3.04	1.00	2.16	1.18	*
inMONO		*		4.51	*	*	3.58	*			*
MAST_CELL	*	*		*		*	*				*
MOMDSC		*		*	*	*	0.41	*			*
MONO		2.53		4.40	*	*	1.68	*			*
ncMONO		*		7.23	*	*	2.84	*			*
NEUTROPHIL	8.94	*		8.70		*	2.93				*
NK	7.39	1.64	*		*		1.09	1.57		1.25	0.39
NKT	*	*	*		*	*	2.86	2.27	2.07	5.29	*
PB	*	*	*			*	*	*			
pDC		*		*		*	1.34				*
T_CELL	7.99	2.63	*		0.32	*	2.55	0.83	2.66	1.11	*
transDC		*		*		*	*				*
TREG	7.21	0.37	*		*	*	2.42	*	2.85	0.66	*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

Table 25: Inter-Run Precision (%CV) of Functional State Marker MCV for Donor 3 (HD6)

Functional State Marker	CCR7	CD11B	CD15	CD161	CD25	CD38	CD74	CD8	CD86	CTLA4	
B_CELL		4.44			3.19		1.30		*		
B_MEM		4.32			3.27		1.07		*		
B_NAIVE		4.52			*		1.45		*		

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

BASOPHIL		9.48	*		6.89						
CD16_NK		9.02		0.38		0.52		6.66	1.85		
CD16neg_NK		10.54		1.05		1.85		*	2.49		
CD4_nonTREG	1.70	3.19		0.26		0.23			0.75	3.15	
CD4_TCM	1.62	3.37		0.08		1.21			*	3.00	
CD4_TEM	2.89	4.86		1.80		*			*	*	
CD4_TEMRA	3.14	6.42		2.33		*			*	*	
CD4_TNAIVE		2.91		0.32		0.28			*	*	
CD56hi_NK		9.71		1.18		0.40		*	3.30		
CD8_T	1.31	4.84		1.22		0.30			0.52	*	
CD8_TCM	2.81	3.56		4.98		1.03			*	*	
CD8_TEM	*	7.81		4.12		2.10			*	*	
CD8_TEMRA	*	10.16		2.92		0.56			*	*	
CD8_TNAIVE		4.12		1.71		0.30			0.52	*	
cDC		5.95					1.26		2.08	*	
cMONO		5.61	*				0.89		7.34	*	
DC		5.55					1.53		2.09	*	
DNT	1.55	5.28		0.41		3.68			*	*	
DPT	5.58	5.57		5.14		4.73			*	*	
GDT	3.28	9.54		0.32		1.23			*	*	
inMONO		10.17	*				0.83		7.78	*	
MAST_CELL		*	*		*						
MOMDSC		3.45	*				1.43		6.83	*	
MONO		5.57	*				0.86		6.81	*	
ncMONO		8.06	*				1.78		5.07	*	
NEUTROPHIL		4.49	6.08		1.17						
NK		9.21		0.29		0.41		6.80	1.79		
NKT	10.31	5.63		0.09		8.79			*	*	
PB		*			*		*		*		
pDC		*					2.28		*	*	
T_CELL	1.64	3.90		0.32		0.22			0.90	2.22	
transDC		*					*		*	*	
TREG	2.96	3.19		3.92		2.14			*	1.16	
Functional State Marker	HLADR	KI67	LAG3	LOX1	PD1	PDL1	RBM39	TBET	TCF1	TIGIT	TIM3
B_CELL	3.74	*	*			*	2.01	2.23			
B_MEM	3.26	*	*			*	2.56	1.84			
B_NAIVE	4.09	*	*			*	1.79	*			
BASOPHIL	*	*		9.03		*	0.88				*
CD16_NK	*	6.46	*		*		0.32	0.35		1.13	*
CD16neg_NK	*	*	*		*		1.04	2.25		4.18	*
CD4_nonTREG	1.64	7.69	*		0.32	*	2.02	0.08	3.03	0.46	*

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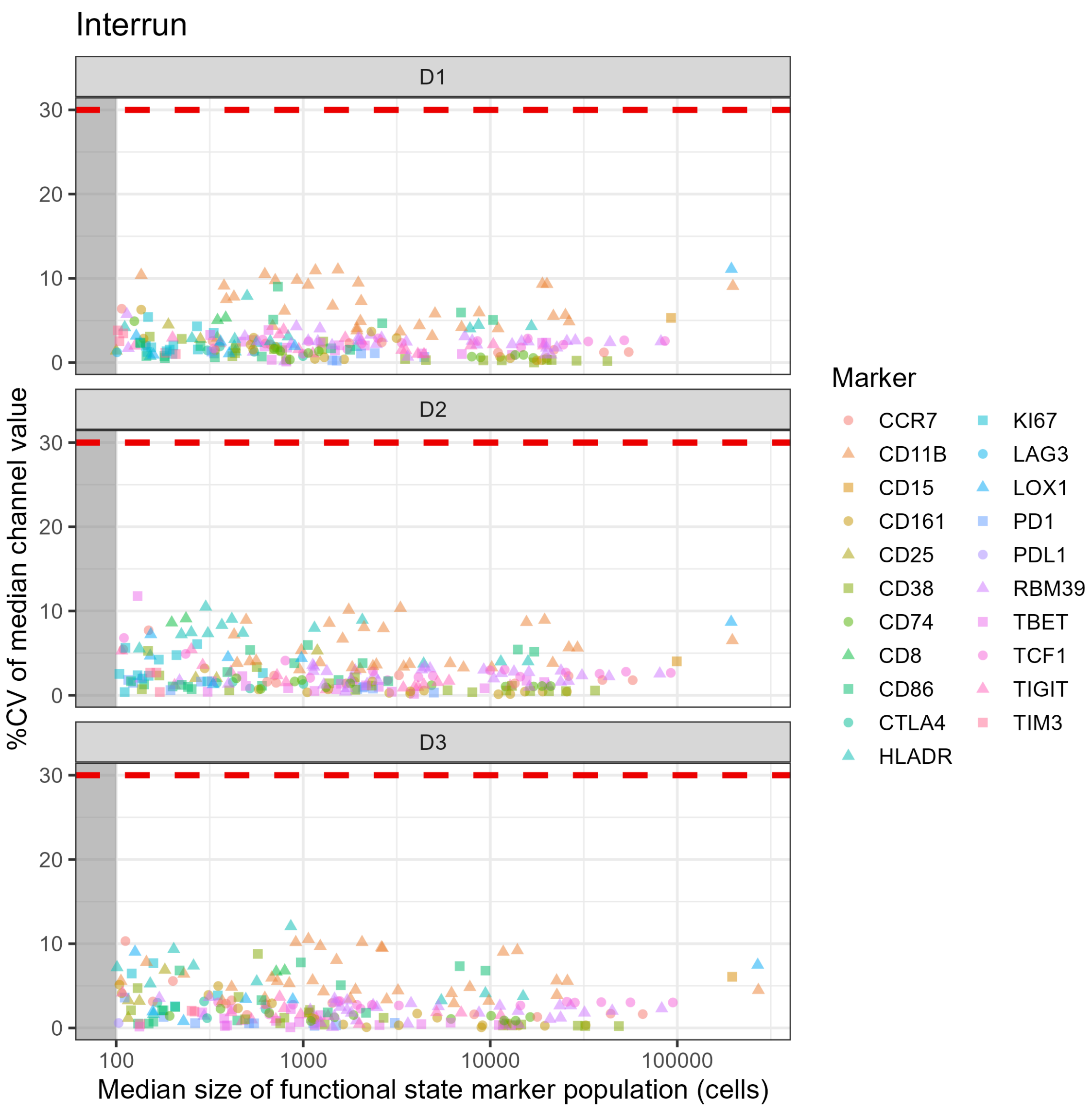
CD4_TCM	*	*	*		0.59	*	2.61	0.30	2.67	0.53	*
CD4_TEM	*	*	*		*	*	2.14	*	3.19	*	*
CD4_TEMRA	*	*	*		*	*	2.73	*	2.76	*	*
CD4_TNAIVE	*	*	*		0.57	*	1.80	0.24	3.06	1.02	*
CD56hi_NK	*	*	*		*		0.61	0.71		*	*
CD8_T	7.38	*	*		0.25	*	2.66	0.63	3.01	1.81	*
CD8_TCM	*	*	*		0.55	*	2.12	1.13	2.74	1.59	*
CD8_TEM	*	*	*		*	*	3.54	0.17	*	*	*
CD8_TEMRA	*	*	*		*	*	3.44	0.37	1.60	2.35	*
CD8_TNAIVE	5.26	*	*		0.56	*	2.65	0.45	3.11	1.85	*
cDC		*		*		*	1.39				2.05
cMONO		*		0.79	*	*	1.06	*			*
DC		*		*		*	1.79				1.94
DNT	*	*	*		1.31	*	2.70	0.84	2.72	3.69	*
DPT	*	*	*		*	*	1.43	*	3.04	*	*
GDT	*	*	*		3.61	*	2.92	0.34	2.67	0.79	*
inMONO		*		3.41	*	*	3.26	*			*
MAST_CELL	*	*		*		*	*				*
MOMDSC		*		*	*	*	0.09	*			*
MONO		*		3.38	*	*	1.21	*			*
ncMONO		*		1.95	*	*	2.96	*			*
NEUTROPHIL	12.05	*		7.47		0.59	0.50				*
NK	9.34	2.57	*		*		0.40	0.33		1.40	0.47
NKT	*	*	*		*	*	2.45	1.52	2.29	1.84	*
PB	*	*	*			*	*	*			
pDC		*		*		*	3.64				*
T_CELL	5.50	3.85	*		0.61	*	2.30	0.52	3.02	1.50	*
transDC		*		*		*	*				*
TREG	7.19	*	*		*	*	2.49	*	3.17	0.99	*

Numbers represent %CV. *Populations in which fewer than two replicates had sufficient cell count (≥ 100) were excluded from CV calculations. Measurement exceeds acceptance criteria is highlighted in Grey.

Figure 10: Coefficient of Variation in Functional State Marker MCV and Median Number of Cells per Functional State Marker Positive Population for Inter-Run Precision Assessment



8.6 Conclusion

Table 26: Summary of Assay Accuracy and Precision

		Median CV [%]	Percentage of populations Pass (CV ≤ 30%)
Intra-run	Immune Cell Frequency of Top Level Gate	1.64	100 (100/100 measurements)

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	Functional State Marker - Frequency of Parent	2.77	99.8 (556/557 measurements)
	Functional State Marker - MCV	0.75	100 (545/545 measurements)
Inter-run	Immune Cell Frequency of Top Level Gate	3.69	100 (101/101 measurements)
	Functional State Marker - Frequency of Parent	7.86	79.2 (483/610 measurements)
	Functional State Marker - MCV	2.17	100 (595/595 measurements)

The ABC001 assay meets the qualification criteria for robustness and reproducibility in measuring immune cell frequencies and their functional states, demonstrating consistent measurements across testing conditions, including both intra-run and inter-run precision assessments.

All measurements in the intra-run precision assessment passed our threshold (CV ≤ 30%), with median %CV at 1.64% for immune cell frequency and 2.77% for functional state marker frequency, calculated from three replicates within the same run across three donors. For the inter-run precision assessment, immune cell frequency measurements also met our acceptance criteria, with a median %CV at 3.69%, calculated from three replicates of three separate runs across three donors.

Regarding functional state marker frequencies, the inter-run precision showed a slightly lower percentage of measurements meeting the precision threshold compared to intra-run results (79% vs. 99%). Among the 21% of measurements with higher variance across three runs, CD86 (38 measurements), CD11B (25 measurements), and CD25 (11 measurements) were the most affected markers. Most other markers consistently met the acceptance criterion across runs with median %CV at 7.86%. This observed variability reflects the inherent differences introduced by operator and instrument conditions over multiple days, indicating that certain functional state markers (or their metal tags) are more sensitive to subtle shifts in staining, gating, or instrument calibration. To achieve optimal precision, we recommend batching samples with expected changes in these functional state markers within the same run or implementing batch normalization to correct for batch-to-batch variability. All measurements of the functional state marker MCV including both intra-run and inter-run precision assessments passed our threshold with median %CV at 0.75% (intra-run) and 2.17% (inter-run).

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.

9. References

Standard Operating Procedures

- DCS006 Preparation of CyTOF reagents
- DCS007 CyTOF Staining with Barcode
- DCS034 Quality Assurance and Performance Verification
- DCS130 CyTOF TokuKit Performance Validation Plan

Manuals

- TokuKit manual

Appendix

- ABC001c gating QC report
- Supplementary tables of precision assessments

10. History block

Revision	Originator	Date Effective	Nature of Change
01	Li-Chun Cheng	2024.10.31	Initial Release

Reviewed by

Name	Date	Signature
Jacek Klepacki	11/1/2024	

The assay has met all predefined acceptance criteria and been qualified as fit-for-purpose for the detailed immune monitoring of clinical samples but is not FDA-approved for diagnostic use.