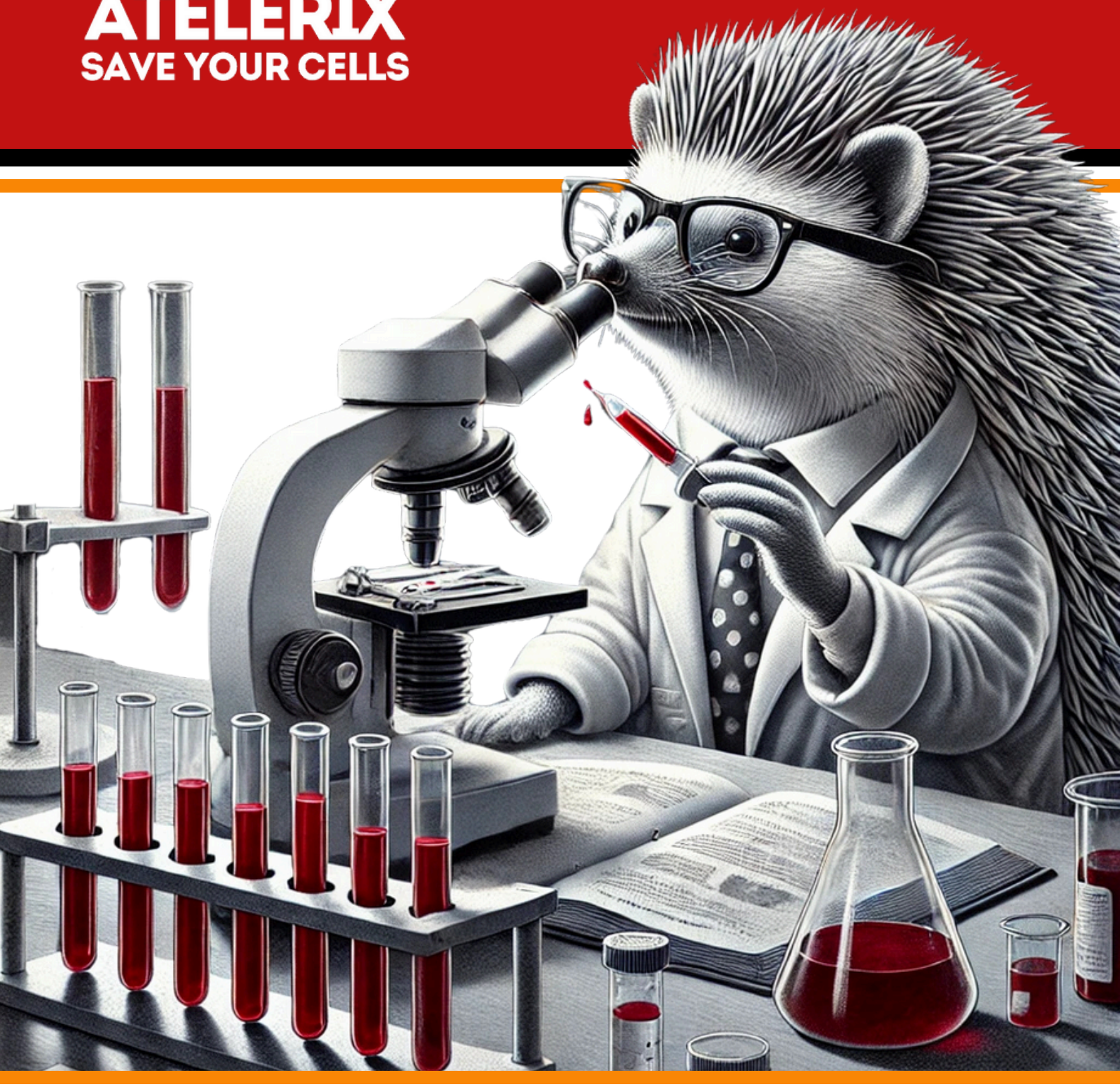
ATELERIX
SAVE YOUR CELLS



BLOODREADY™
white paper

fresh whole blood
shelf life amplified!!!

BLOODREADY

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1.0 INTRODUCTION

This report provides data to support the storage and transport of human whole blood by our technology, BloodReady™. Immunophenotyping whole blood specimens by flow cytometry is an incredibly powerful tool in the assessment of human health and is used widely in clinical patient diagnosis and monitoring (1). Alongside it's clinical use, immunophenotyping allows epidemiological prediction of disease risk, and has aided in the development and implementation of multiple immunotherapies.

Flow cytometric immunophenotyping is conducted at analysis centres, which are often distinct from the site of whole blood collection, and therefore transportation of samples is required (2). The processing of diagnostic samples can however be delayed by transportation, as well as by personnel and equipment availability. Since whole blood stability is limited after 24h following collection (3) (4), this can have several consequences for the outcome of flow cytometric analysis. Prolonged blood storage leads to selective loss of cell populations with a short half-life time, such as eosinophils and neutrophils (5) (6). Additionally, biochemical changes, such as decrease in pH, changes in blood gases (7), alterations in the contents of amino acids, carbohydrates, lipids and cofactors (8) (9), might further influence cell viability.

As same-day processing of whole blood samples is not always feasible, researchers have implemented strategies to extend the time to analysis. This includes various fixative reagents including TransFix, Cyto-Chex, and formaldehyde, cryopreservation, and isolation of PBMCs (10) (11) (12). While these strategies permit increased flexibility in workflow, whole blood fixation is reported to compromise the relative distribution of leukocytes, leading to under and over representation of certain populations (13) (14). Furthermore, these fixative reagents show negative impact on marker expression (15), leading to impaired discrimination of leukocyte subsets over time. Such harm to leukocyte cell quality renders whole blood samples unsuitable for detailed immunophenotyping and in turn clinical utility.

Atelerix's technology, BloodReady™, offers a solution to overcome these logistical challenges of whole blood processing and transportation, without limiting the quality and informativeness of data obtained. Another significant advantage to BloodReady™ sample preservation is the ability to batch process samples, which enhances the consistency and quality of the analysis and can help in the management of programs, costs and timelines.

2.0 METHODS

2.1 SAMPLE ACQUISITION

Throughout the methods herein, whole blood samples were obtained from healthy volunteers. Whole blood was collected into Sodium Heparin Vacutainer Tubes; whole blood leukocyte populations are reported to be more stable in Sodium Heparin compared to EDTA (2). Two different conditions were tested: non-stored blood and BloodReady-stored blood for 3 days at 2-8°C. A volume of 3 mL whole blood was used per condition.

2.2 BLOODREADY™ STORAGE & RELEASE

For storage in BloodReady, BloodReady beads had been prepared in 10 mL collection tubes. For each encapsulation, 3 mL blood was mixed with 0.75 mL of Gel A. The blood and Gel A mixture was added to the BloodReady beads slowly and inverted once to allow adequate mixing of the beads and blood. The sample was then left for 15 minutes at RT to allow for gelation to occur. Following encapsulation, the BloodReady samples were stored at 2-8°C for 3 days.

To release the encapsulated whole blood, 6 mL of dissolution buffer (0.2M Citrate) was added to the bottom of the sample tube to dislodge the beads. The beads were left to dissolve for 15 minutes at RT on the rocker at 40rpm. Released blood samples were then filtered to remove dissolved beads by pipetting the blood sample through a 100 µm filter into a 50 mL falcon tube. The released sample was then used for subsequent downstream application. For PBMC isolation, SepMate tubes were used according to manufacturer's instructions (STEMCELL Technologies).

2.3 MULTIPLEX ANTIBODY STAINING

For multiplex antibody staining of blood immune cells, 100 µL of non-stored whole blood, or 150 µL of released stored blood, was added to a 5 mL Falcon™ Round-Bottom Polypropylene Test Tube. A 33-colour antibody panel was then added to the blood (see Supplementary Table 1 for list of antibodies). The falcon tube was then left for blood immune cell surface epitope staining for 30 minutes at RT. Following staining, 2 mL of warmed PharmLyse solution was then added and the falcon tube was incubated for 20 minutes at RT to allow RBC-lysis to occur. Following lysis, the falcon tube was spun down at 500xg for 5 minutes at RT. The residual volume as aspirated and discarded, avoiding the cell pellet. 2 mL of wash buffer was added to the blood sample and the falcon was spun down at 500xg for 5 minutes at RT. The residual volume was removed, avoiding the cell pellet, and cells were resuspended in 300 µL of wash buffer. Samples were then run on Northern Lights to analyse immune cell populations. Data was compared between Day 0 non-stored blood and 3 Day BloodReady preserved blood. Data was analysed using FCS Express Software.

2.4 EPITOPE EXPRESSION ANALYSIS

Whole blood was stored for 3 days at 2-8°C in either BloodReady™ or major competitor fixative reagent Cyto-Chex, and released as described in 2.2. For staining of non-stored blood leukocyte surface epitopes, 100 µL of non-stored whole blood was added to 50 wells of 2 x 96-deep well plates. For stored blood, 150 µL of BloodReady™ preserved blood was added to 50 wells of 2 x 96-deep well plates, and 100 µL of Cyto-Chex preserved blood was added to 50 wells of 2 x 96-deep well plates. A CD45 antibody was then added to each well. The corresponding PE antibody of the 93-antibody panel was then added to the corresponding well (see Supplementary Table 2 for list of antibodies). The 96wp was then left for blood surface epitope staining for 30 minutes at RT. Following staining, 2 mL of warmed PharmLyse solution was then added and the plates were incubated for 20 minutes at RT to allow RBC-lysis to occur. Following lysis, the plates were spun down at 500 xg for 5 minutes at RT. The residual volume was aspirated and discarded, avoiding the cell pellet. 2 mL of wash buffer was added to each well and the plates were spun down at 500 xg for 5 minutes at RT. The residual volume was aspirated and discarded, avoiding the cell pellet. 2 mL of wash buffer was added to each well and the plates were spun down at 500 xg for 5 minutes at RT. The residual volume was removed, avoiding the cell pellet, and cells were resuspended in 300 µL of wash buffer. Samples were then run on Northern Lights to analyse epitope expression. Data was compared between Day 0 non-stored blood, 3 Day BloodReady-preserved blood and 3 Day CytoChex preserved blood. Data was analysed using FCS Express Software.

2.5 WHOLE BLOOD IMMUNOPHENOTYPING & POST-REST FUNCTION

Whole blood from healthy donors was stored with or without BloodReady™ at 2-8°C before assessing cell recovery and quality at day 3. The no-gel control (NGC) group represented non-manipulated whole blood. Freeze-thaw samples were preserved in 1ml cryovials with CS10 cell freezing medium. Following cell release at day 3, recovery and viability were examined by trypan blue exclusion. Whole blood immunophenotyping was performed by flow cytometry staining on 100µl of blood for CD45+ (all leukocytes), CD3+ (T cells), CD19+ (B cells), CD56+ (NK cells), CD14+ (monocytes), and CD16+ SSChi (neutrophils). Viability was assessed by Annexin V and 7AAD staining; live (unstained), apoptotic (AnV+) and apoptotic (7AAD+) cells. In parallel, SepMate™ PBMC Isolation Tubes were used to isolate PBMCs from released whole blood samples. PBMCs were cultured overnight to assess post-rest recovery and viability via trypan blue exclusion, and IL-2 stimulatory response. Cells were stimulated with 600 IU/mL IL-2 upon return to culture, and PBMC counts were assessed by trypan blue exclusion after 2-3 days.

3.0 RESULTS

3.1 PRESERVATION OF WHOLE BLOOD IMMUNE CELL POPULATIONS USING BLOODREADY™

A 33 Colour Panel was used to test the preservation of whole blood leukocyte populations after storage in BloodReady™. Multiplex staining allowed comprehensive delineation of immune cell populations including rare immune cell subsets (See Figure 2 for hierarchy). Whole blood was collected from 4 healthy volunteers and stored for 3 days at 2-8°C in BloodReady™. Non-stored whole blood was tested on Day 0, and then after 3 days' storage in BloodReady™.

An initial assessment of leukocyte viability comparing non-stored blood and BloodReady ™-preserved blood showed no significant difference in the proportion of live leukocytes (Figure 1) between conditions. In non-stored blood, viable leukocytes represented 91.8% ± 1.4 of the total CD45+ population, compared to 94% ± 1.7 in BloodReady-preserved blood.

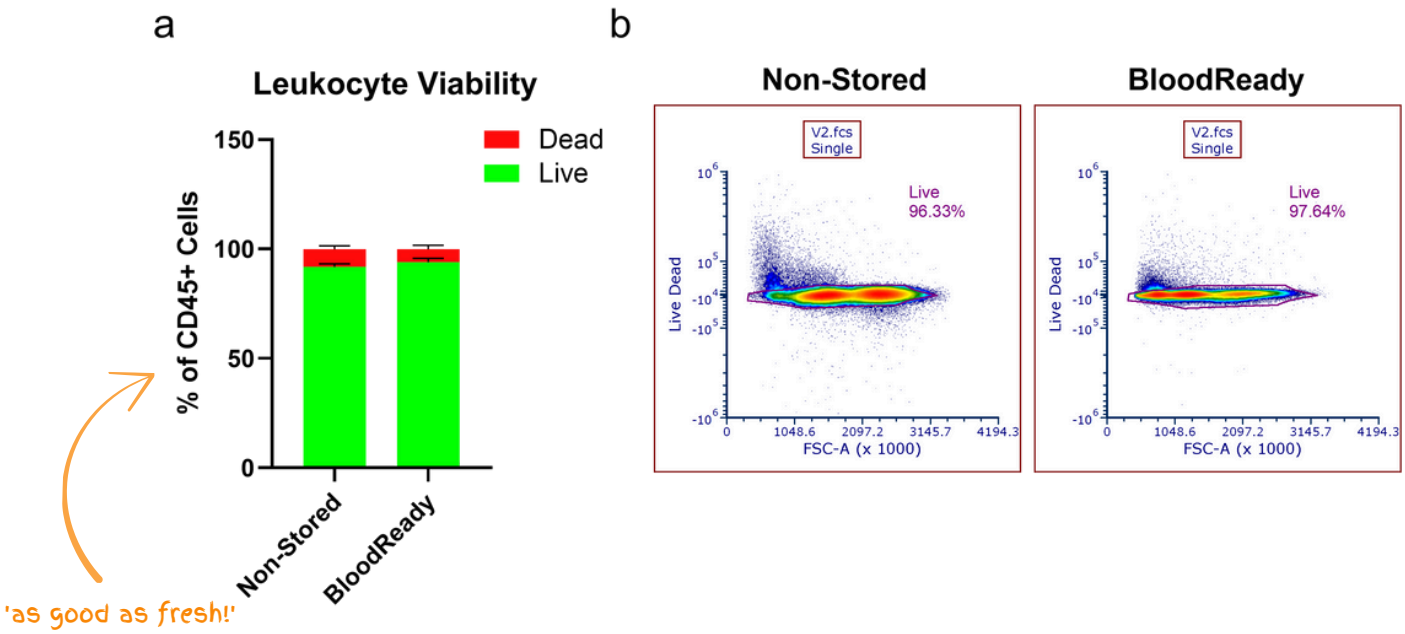


Figure 1. Preservation of whole blood leukocyte viability using BloodReady™. *Total single cells were firstly defined as leukocytes (CD45+), and subsequently identified as live or dead using the LIVE/DEAD™ Fixable Blue Dead Cell Stain. (a) Viable CD45+ cell count was expressed as a percentage of total CD45+ cells. (b) Representative plot of non-stored and 3-day BloodReady™-stored blood from one healthy volunteer.*

Comprehensive immunophenotyping by multiplex antibody staining showed that BloodReady™ preserves the relative proportions of major circulating leukocyte populations including T cells, NK cells, B cells, and monocytes (Figure 3a). T cells (CD3+) increased by 4.3%, B cells (CD19+) by 4.5%, NK cells (CD56+ CD16+) by 1.6%, Lymphoid Cells (ILC) (CD127+) by 0.14%, and Monocytes (CD4+ HLA-DR-) by 4.1%. Further interrogation of these major leukocyte populations was then performed to assess any impact of BloodReady™ preservation on intricate and rare immune cell populations.

Delineation of the T cell population (Figure 3b) shows that Helper T cells (CD4+) increase by 3.3%, Cytotoxic T cells (CD8+) increase by 1.1%, Regulatory T cells (CD25+ CD127-/dim) decrease by 0.2%, while Gamma delta T cells (TCRγδ+) and Natural Killer T cells (CD56+) remain unchanged after 3-day storage in BloodReadyTM. Delineation of the B cell population (Figure 3c) shows that Transitional B cells (CD27- IgD+) increase by 3.8%, Non-Switched B Cells (CD27+ IgD+) increase by 0.3%, and Switched B cells (CD27+ IgD-) increase by 0.2%.Subdivision of NK cells (Figure 3d) showed that CD57+ NK cells decrease by 0.2%, Early NK cells (CD56+ CD16-) remain unchanged, and Mature NK cells (CD56+ CD16+) increase by 1.2%. Subdivision of monocytes (Figure 3e) show that Classical Monocytes (CD14+ CD16-) increase by 3.6%, Intermediate Monocytes (CD14+ CD16+) decrease by 0.02%, and Non-Classical Monocytes decrease (CD14- CD16+) by 0.06%. Lymphoid Cells (ILC) (CD127+) were subdivided (Figure 3f) into ILCs CD2+ CD4+, and ILCs CD2+ CD4- which show increases of 0.1% and 0.06%, respectively. Dendritic cells were identified as HLA-DR+ CD14- cells and subdivided into CD11c+ DCs and CD123+ pDCs (Figure 3g). CD11c+ DCs showed an increase of 0.27% while CD123+ pDCs also showed a negligible increase of 0.019% after storage in BloodReady™. Granulocytes, which encompass Neutrophils, Eosinophils, and Basophils are the only population that show a significant change in their proportion after storage in BloodReady™. Granulocytes decrease by 18.6% compared to non-stored blood, suggesting a sensitivity of this cell type to the encapsulation preservation process.

BloodReady™ maintains live populations of T Cells, B Cells, NK Cells, ILCs, monocytes, and their respective cell subsets, after 3 days' storage at 2-8°C. The accurate delineation of major leukocytes into immune cell subpopulations shows that important cell markers that are used in the characterisation of these cells are retained after storage in BloodReady™. This in turn allows successful flow cytometry applications on whole blood after storage in BloodReady™, for use in immunophenotyping, cell monitoring, and diagnosis.

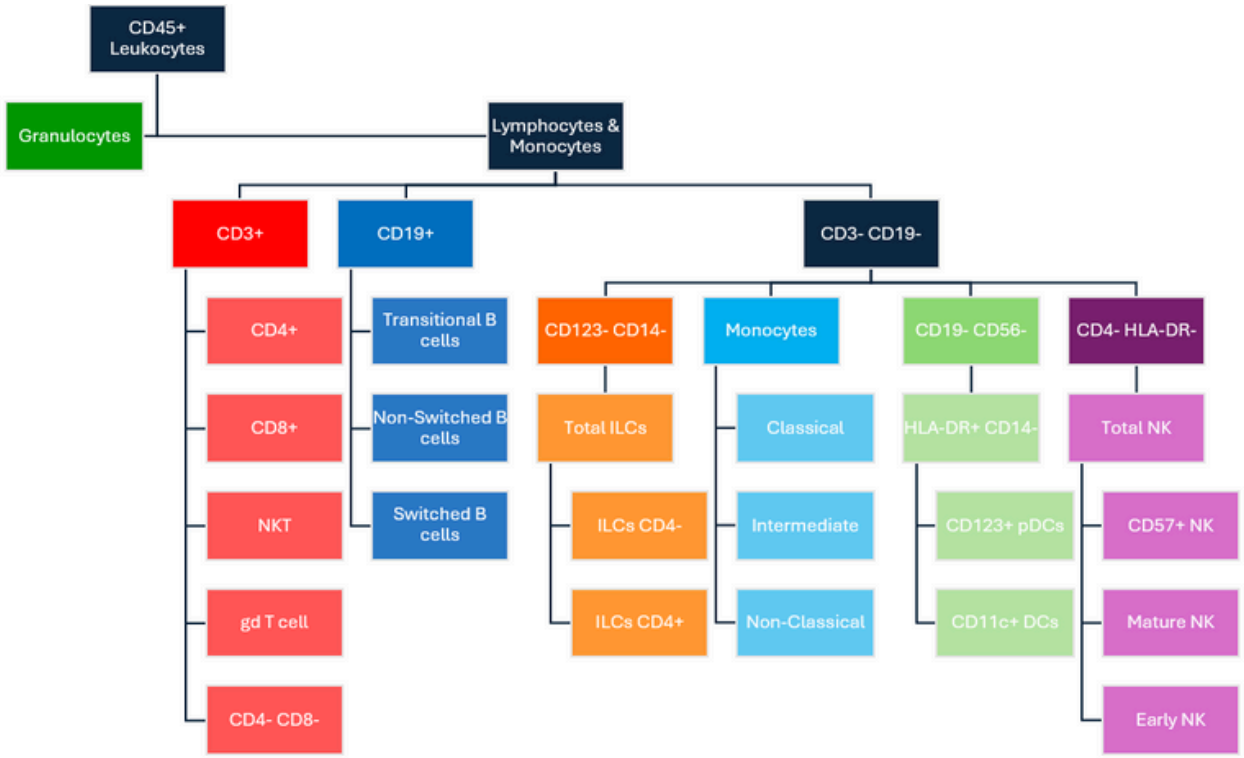


***Figure 2**. Hierarchy to show delineation of immune cell subsets by multiplex flow cytometry antibody staining.*

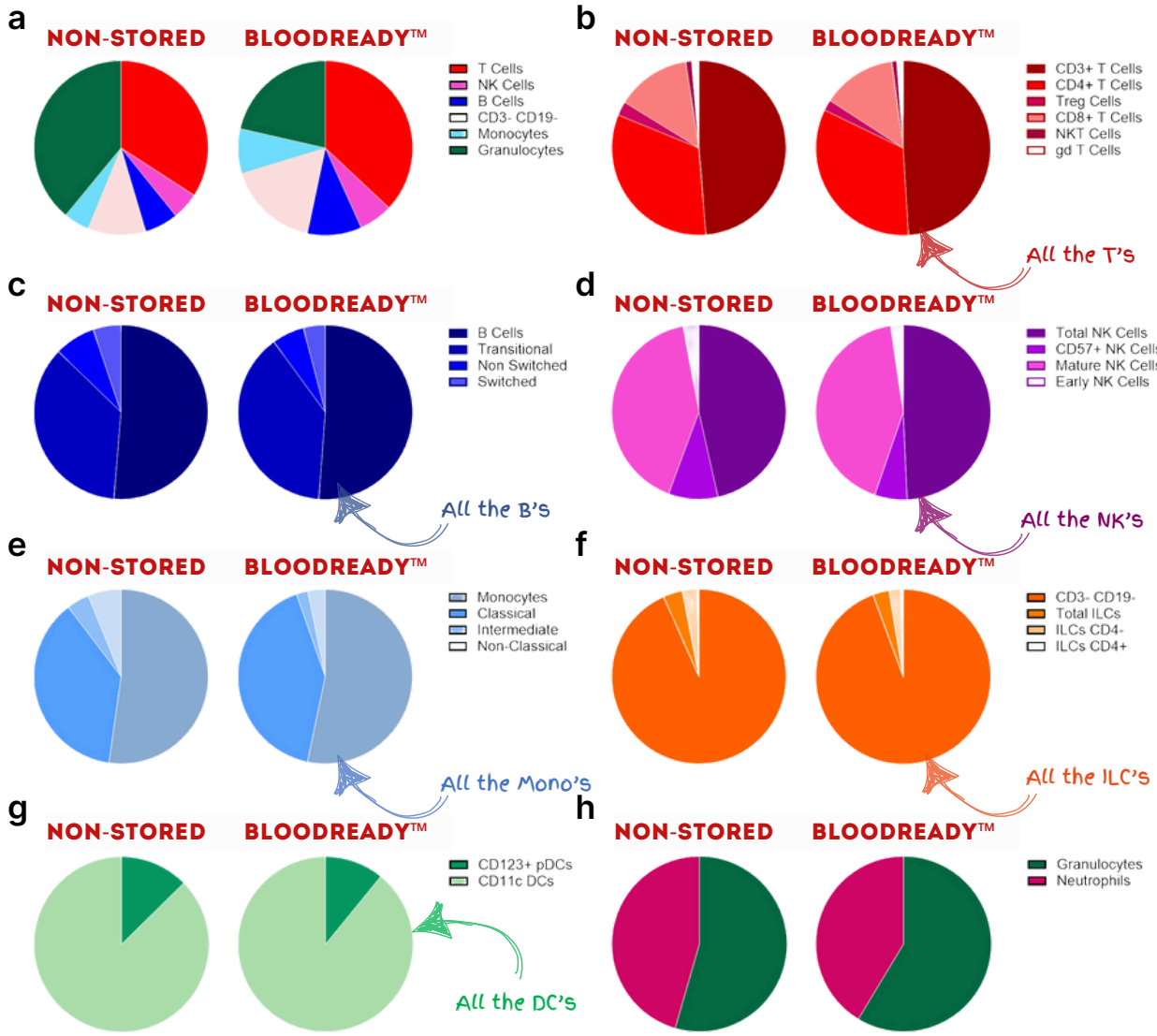
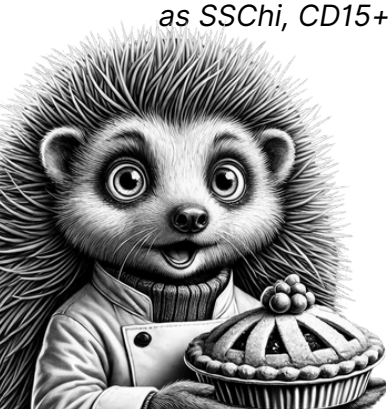


Figure 3. Preservation of whole blood immune cell populations using BloodReady™. *Total single cells were firstly defined as leukocytes (CD45+), and subsequently identified as live or dead using the LIVE/DEAD™ Fixable Blue Dead Cell Stain. a) Immune cell subpopulations are expressed as a percentage of total live CD45 cells. b) T Cells (CD3+) were defined and delineated into Helper T cells (CD4+), Cytotoxic T cells (CD8+), Regulatory T cells (CD25+ CD127-/dim), Gamma delta T cells (TCRγδ+), and Natural Killer T cells (CD56+). c) B Cells (CD19+) were defined and subdivided into Transitional B cells (CD27- IgD+), Non Switched B Cells (CD27+ IgD+), and Switched B cells (CD27+ IgD-). d) NK Cells (CD56+ CD16+) were defined and subdivided into CD57+ NK cells, Early NK cells (CD56+ CD16-), and Mature NK cells (CD56+ CD16+). e) Monocytes were defined (CD4+ HLA-DR-) and subdivided into Classical Monocytes (CD14+ CD16-), Intermediate Monocytes (CD14+ CD16+), and Non-Classical Monocytes (CD14- CD16+). f) Innate Lymphoid Cells (ILC) were defined as CD127+, and subdivided into ILCs CD2+ CD4+, and ILCs CD2+ CD4-. g) Dendritic cells were defined as CD11c DCs and CD123+ plasmacytoid DCs. h) Granulocytes were defined as SSChi, CD15+ CD11b+ cells. For the antibody panel, see Supplementary Table 1.*



"preservation of both the major players and rarest immune cell populations is remarkable"

Immune Cell Population	Non-Stored % of CD45+ Cells	Non-Stored SD	BloodReady % of CD45+ Cells	BloodReady SD	Difference (%)	Significance
Live Leukocytes	91.77	± 1.42	93.98	± 1.67	2.21	ns
T Cells	37.60	± 7.61	41.95	± 4.65	4.34	ns
CD4+ T Cells	25.05	± 4.36	28.36	± 4.32	3.31	ns
T reg Cells	1.99	± 0.37	1.70	± 0.11	-0.29	ns
CD8+ T Cells	10.68	± 4.22	11.77	± 4.27	1.09	ns
NK T Cells	0.83	± 0.74	0.78	± 0.98	-0.05	ns
γδ T Cells	1.02	± 0.66	0.99	± 0.66	-0.03	ns
B Cells	6.87	± 2.39	11.40	± 4.42	4.53	ns
Transitional B Cells	4.81	± 2.18	8.64	± 3.88	3.82	ns
Non-Switched B Cells	1.01	± 0.35	1.33	± 0.52	0.31	ns
Switched B Cells	0.69	± 0.17	0.90	± 0.26	0.21	ns
NK Cells	5.53	± 1.89	7.09	± 2.43	1.56	ns
CD57+ NK Cells	1.09	± 1.22	0.86	± 1.15	-0.23	ns
Mature NK Cells	4.93	± 1.94	6.11	± 2.49	1.18	ns
Early NK Cells	0.34	± 0.13	0.32	± 0.15	-0.02	ns
Monocytes	5.24	± 1.81	9.37	± 2.76	4.13	ns
Classical Monocytes	3.75	± 1.41	7.28	± 2.15	3.53	ns
Intermediate Monocytes	0.42	± 0.14	0.37	± 0.04	-0.05	ns
Non-Classical Monocytes	0.62	± 0.50	0.56	± 0.41	-0.06	ns
Innate Lymphoid Cells (ILCs)	0.45	± 0.10	0.61	± 0.18	0.16	ns
CD4- ILCs	0.34	± 0.10	0.42	± 0.17	0.08	ns
CD4+ ILCs	0.04	± 0.02	0.10	± 0.04	0.06	ns
pDCs CD123+	0.08	± 0.03	0.10	± 0.04	0.02	ns
CD11C DCs	0.59	± 0.58	0.86	± 0.63	0.27	ns
Granulocytes	42.97	± 9.67	24.28	± 5.96	-18.69	ns
Neutrophils	35.91	± 10.34	17.13	± 7.52	-18.78	ns

Table 1. Preservation of whole blood immune cell populations using BloodReady™. *Data described in Figure 3 was analysed for significance using the Wilcoxon matched pairs signed rank test (paired, non-parametric). Non-significant (ns) = p > .05.*

"wow, there's hardly any % change in any of the live cell populations versus fresh blood...the granulocytes get in the way anyway!"

3.2 PRESERVATION OF LEUKOCYTE EPITOPE PRESENTATION

Populations of cells are defined based on the presence or absence of specific markers and their expression levels. This is particularly important in disease states whereby leukocyte proportions are disturbed, or marker expression is dysregulated. For accurate clinical diagnosis and monitoring, it is therefore vital that the expression of cell markers at the time of analysis is representative of whole blood leukocytes in vivo. This is often a challenge, as studies have demonstrated that discrimination of cell populations in non-stored whole blood is increasingly impaired over time (2), thereby limiting the utility of blood samples after 24h from collection. Fixative reagents including Cyto-Chex, TransFix, and formaldehyde can extend the storage time of whole blood prior to flow cytometric analysis, however they can also have deleterious effects on marker expression.

We investigated the ability of BloodReady™ to preserve key epitopes expressed on the surface of blood leukocytes compared to the fixative reagent Cyto-Chex. Whole blood was stored for 3 days at 2-8°C in either BloodReady™ or major competitor fixative reagent Cyto-Chex. After release, leukocyte epitope expression was evaluated by staining with a panel of 93 antibodies against leukocyte-surface markers. Leukocytes were discriminated into subpopulations of lymphocytes, monocytes, and granulocytes to assess cell type-specific epitope preservation. Epitope expression was measured and expressed as a ratio of that in non-stored, where 0 = epitope expression equal to non-stored.

We show that BloodReady™ preserves the expression of >90% epitopes screened on lymphocytes (Figure 4a), >80% on monocytes (Figure 4b) and >70% on granulocytes (Figure 4c). Comparison with major competitor fixative solution Cyto-Chex showed that BloodReady™ preferably preserved several epitopes including CD62L, CD317, CD82, CD367, and integrin b7 (Figure 4d). Preservation of CD62L is particularly useful given it's reported sensitivity to cryopreservation methods. BloodReady™ offers a simple and effective solution for preserving whole blood leukocyte populations and their respective immunophenotype profile for up to 3 days. This solution is advantageous over Cyto-Chex, providing improved preservation of markers up to 72h. The downstream applications of whole blood preserved by BloodReady™ are unlimited, unlike fixative solutions which restrict subsequent functional assays.

This data has important implications for preclinical immune cell assessment, clinical diagnostics, and clinical monitoring, all of which rely on reproducible and accurate immunophenotyping data. Importantly, BloodReady™ offers a solution to avoid the disadvantages of cryopreservation and fixing solutions.

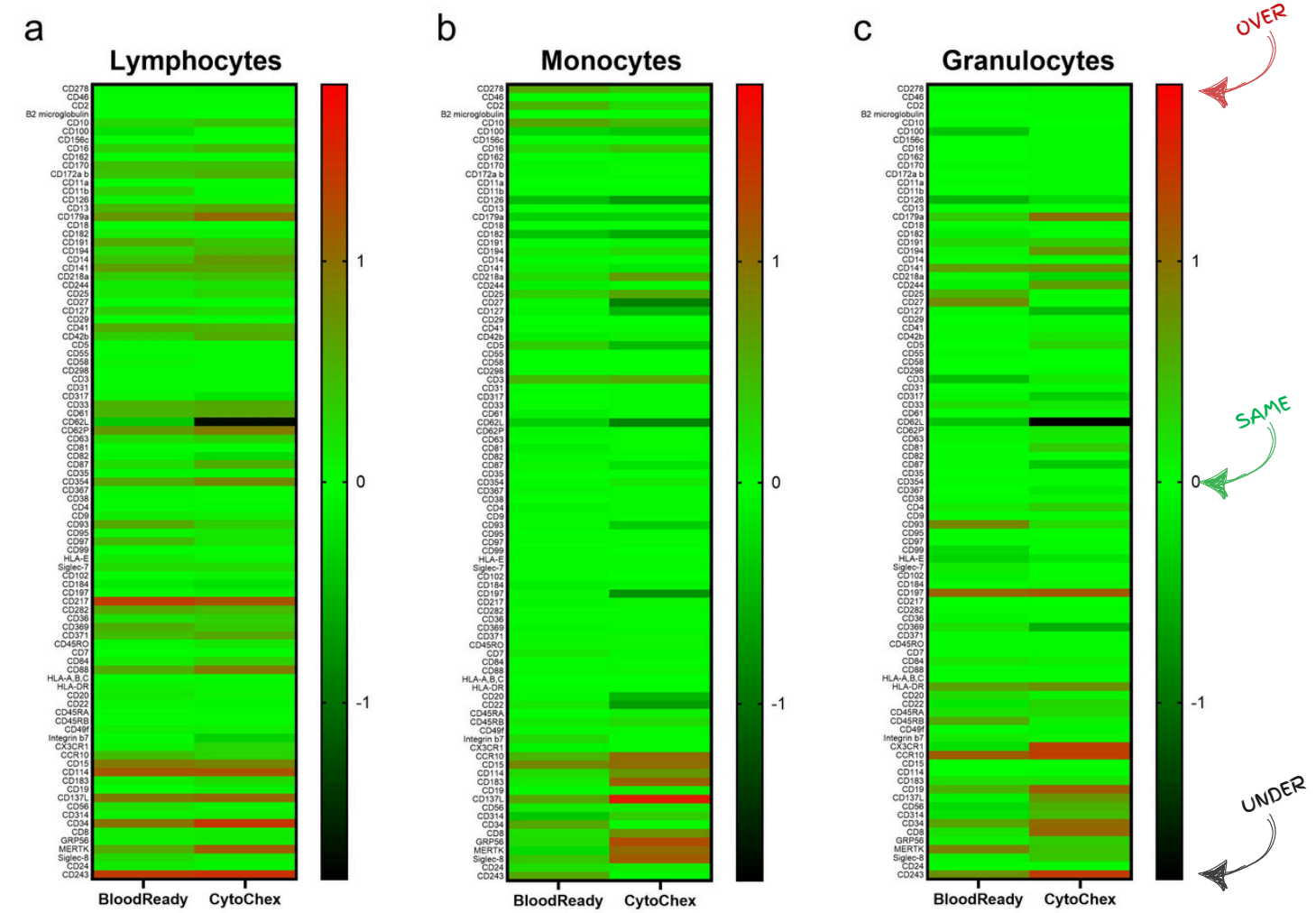


Figure 4. Preservation of whole blood leukocyte epitope expression by BloodReady™. *Whole blood was stained with a panel of 93 antibodies against leukocyte surface epitopes following 3-day storage in BloodReady™ or Cyto-Chex. Epitope expression was analysed against that of non-stored whole blood leukocytes and expressed as a ratio, where 0 = non-stored. Leukocyte epitope expression was analysed separately for a) lymphocyte, b) monocyte, and c) granulocyte cell subpopulations. d) BloodReady™ preserves several lymphocyte epitopes more effectively than Cyto-Chex, including CD62L, CD317, CD82, CD367, and Integrin b7. Data shows the mean of n=3 healthy donors. For the antibody panel, see Supplementary Table 2.*

'75% epitopes preserved better than Cyto-Chex...exceptional epitope stability!'

3.3 ISOLATING AND CULTURING LIVE PBMCS FROM STORED WHOLE BLOOD

Whole blood leukocyte recovery following 3-day storage in BloodReady™ was assessed and compared with a no-gel control (NGC) and cryopreservation (Figure 5). Whole blood samples were stored at a volume of 3ml. The total viable leukocyte count at day 3 was determined by trypan blue exclusion. We show that storage using BloodReady™ at 2-8°C maintains >95% viability of whole blood leukocytes, out-performing both the NGC and cryopreservation which preserve 75% and 12% leukocyte viability, respectively (Figure 5a).

Comprehensive immunophenotyping of whole blood leukocyte subpopulations was performed in parallel by Flow Cytometry staining of CD45+ leukocytes, CD3+ T cells, CD19+ B cells, CD56+ NK cells, CD14+ monocytes, and CD16+SSChi neutrophils (Figure 5b). Raw leukocyte subpopulation counts were then determined by extrapolating flow cytometry cell subset percentages to the total leukocyte count.

We show that the recovery of viable B cells, T cells, NK cells, and monocytes after 3 days' storage in BloodReady™ yielded cell counts comparable to that of non-stored whole blood leukocytes. BloodReady™ preserved higher viable counts of B cells, T cells, and NK cells compared to the NGC. Cryopreservation of whole blood yielded poor recovery of all leukocyte subpopulations studied.

"live cells can be isolated from the stored blood as well?!?!"

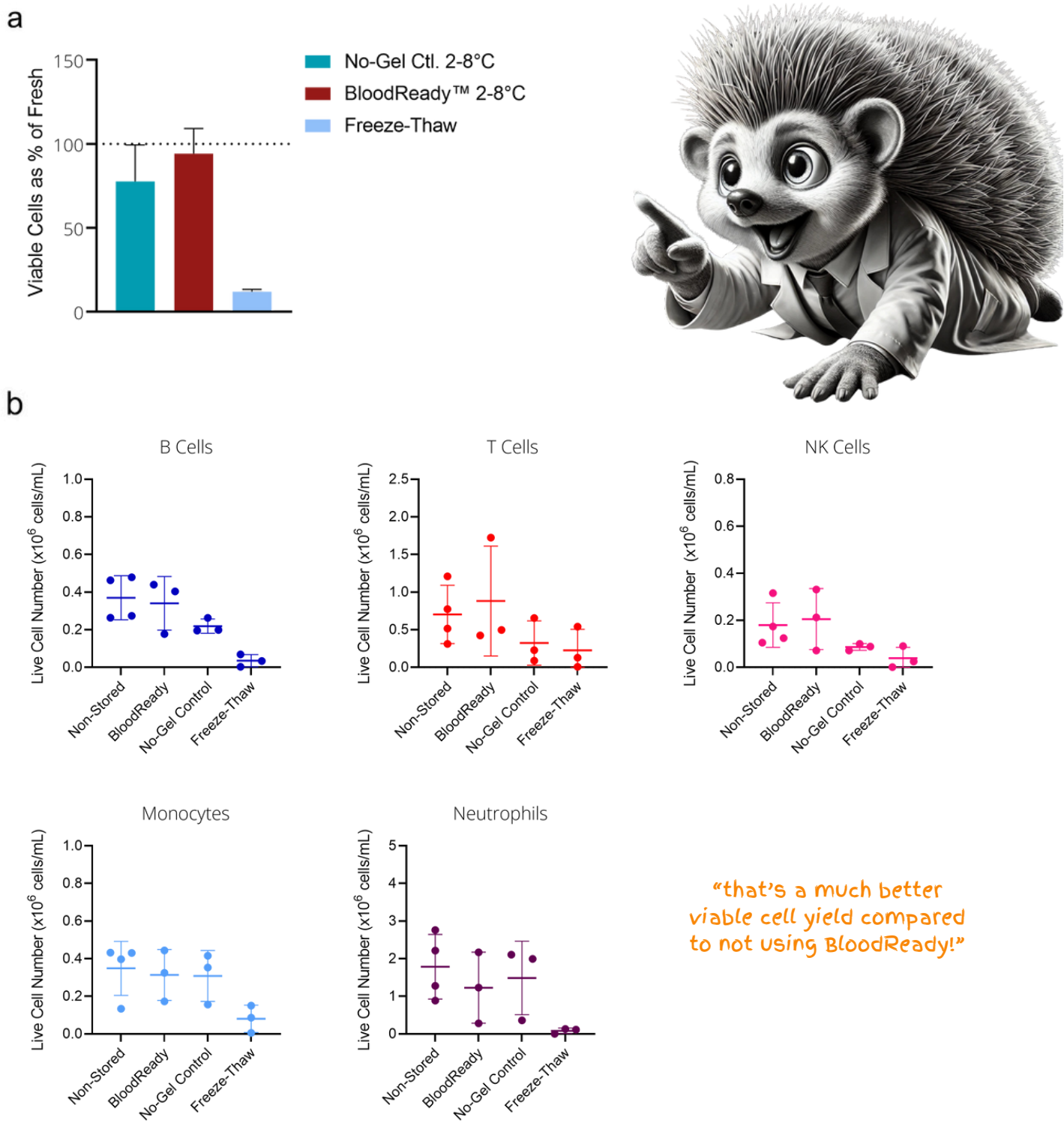


Figure 5. Whole blood preservation using BloodReady™. *a) Recovery of whole blood leukocytes after 3 days storage in BloodReady™ compared to a no-gel control and cryopreservation. b) Recovery of major leukocyte subpopulations following comprehensive immunophenotyping of stored whole blood. Data was obtained from Flow Cytometry staining of all major leukocyte populations. Mean ± SD. Non-Stored: n=4, BloodReady, No-Gel Control, Cryopreservation: n=3*

3.4 RECOVERY AND FUNCTION OF PBMCS FOLLOWING BLOODREADY™ STORAGE

The quality of whole blood leukocytes after 3 days storage in BloodReady™ was evaluated by culturing whole blood isolated PBMCs overnight and assessing both post-rest viability and response to IL-2 cytokine stimulation.

We show that after 24h in culture, BloodReady™ preserved PBMCs retain high viability (>100%), which is comparable with the recovery of non-stored PBMCs. The NGC does not adapt as successfully to the culturing environment, showing a lower viability, suggesting that a deterioration in cell competence is prevented by BloodReady™. Of the few cells that do survive after freezing (see Figure 6a), most recover well, however the original yield is too poor for subsequent downstream application. PBMCs isolated from BloodReady-stored blood also demonstrate a response to IL-2 stimulation after 2-3 days in culture (Figure 6b). In the absence of BloodReady™ storage in the no-gel control, cells do not recover well or respond to IL-2 stimulation. The observed viable cell recovery and IL-2 response of isolated PBMCs after overnight rest suggests that BloodReady™ is a suitable storage solution for subsequent PBMC isolation and application.

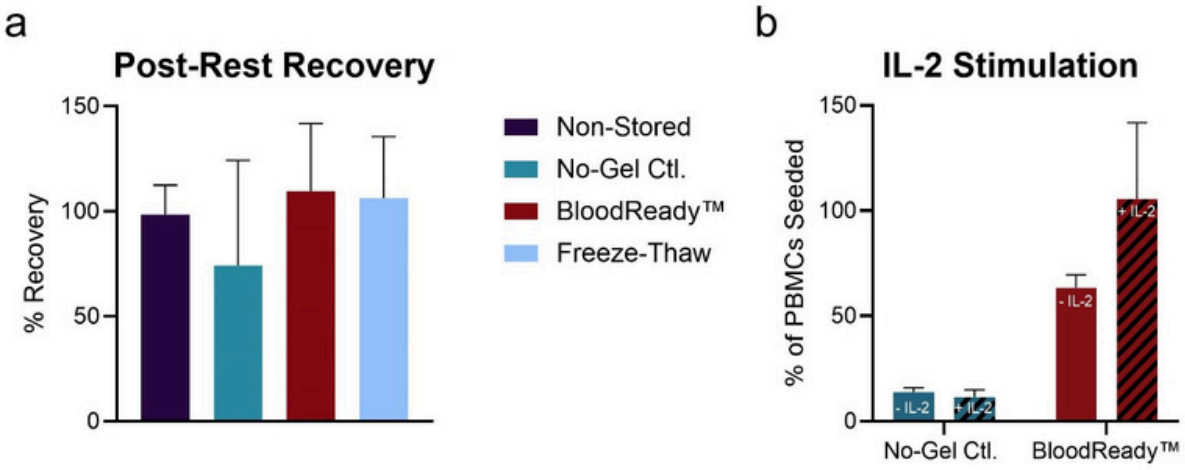


Figure 6. Data shows the post-rest recovery and cytokine stimulatory response of PBMCs in culture after 3 days storage in BloodReady™ at 2-8°C. *a) PBMCs were cultured at 37°C and viability assessed via trypan blue exclusion after 24h in culture. Cell recovery is calculated as cell count as a percentage of cell number seeded. Mean ± SD. Non-Stored: n=3, BloodReady™, No-gel Ctrl: n=4, Cryopreservation: n=2. b) PBMCs were stimulated with/without 200IU IL-2 cytokine for 3 days followed by assessment of cell proliferation by trypan blue exclusion. Mean ± SD. No-gel Ctrl: n=3, BloodReady™: n=3.*

"Voilà, the cells are competent and responsive in culture!"

4.0 CONCLUSION

Improving the preservation of human whole blood opens valuable opportunities for a wide range of clinical and scientific applications. Preservation in the viable state is critical for diagnostic tests and correct patient management. A current major challenge that biopharma and clinical research organisations face is post-sample collection whole blood stability. Sample logistics are complex, as there is a need to consider the time taken to ship the samples from the clinical investigation sample collection sites to the testing facility and the operational requirement to batch process samples. This is particularly important for flow cytometry assays supporting clinical trials whereby accurate cell phenotyping is imperative. Several whole blood stabilization products (e.g., Cyto-Chex and Transfix) have been developed to extend sample stability, however studies report that these measures may not be suitable for all leukocyte populations possibly due to their ability to denature certain epitopes (16) (17), leading to suboptimal detection of certain cellular marker expression. In addition, conflicting results have been documented where these products have failed to provide stability for delayed analysis (2) (18).

This report describes Atelerix's latest technology, BloodReady™, optimised for the storage and transport of human whole blood specimens. BloodReady™ allows extended storage of whole blood at 2-8°C for 3 days while preserving sample integrity and cellular markers for high quality clinical study sample analysis.

5.0 SUPPLEMENTARY

Table 1. Multiplex Staining Antibody Panel

Company	Cat	Marker	Clone	Fluorophore	Mean Peak Channel	Role
BD BioSciences	569489	CD45RA	5H9	HUV395	UV2	T cell differentiation
Thermo	L34961	Viability		Live Dead Blue	UV6	Viability
BD BioSciences	569674	HLA DR	G46-6	BUV496	UV7	Monocyte and T cell activation, NK cell discrimination
BD BioSciences	612994	CD8	SK1	BUV615	UV10	CD8 T cells
BD BioSciences	612967	CD11c	B-ly6	BUV661	UV11	Dendritic differentiation, Monocytes
BD BioSciences	612794	CD127	HIL-7R-M21	BUV737	UV14	T cell differentiation, Treg gating
BioLegend	329920	PD-1 (CD279)	EH12.2H7	BV421	V1	T cell exhaustion
Thermo	48-1619-42	CD161	HP-3G10	eFluor 450	V3	T cell and NK differentiation
BD BioSciences	566138	IgD	IA6-2	BV480	V5	B cell differentiation
BioLegend	317332	CD3	OKT3	BV510	V7	Pan T cell
Thermo	MHCD2030	CD20	HI47	Pacific Orange	V8	B cell differentiation
BioLegend	302036	CD16	3G8	BV570	V8	Monocyte and NK cell differentiation
BioLegend	301332	CD11b	ICRF44	BV605	V10	Monocytes
BioLegend	306020	CD123	6H6	BV650	V11	Plasmacytoid dendritic cells
BioLegend	300232	CD2	RPA-2.10	BV711	V13	NK cell differentiation
BD BioSciences	568933	CD15	7C3	BV750	V14	Neutrophils
BioLegend	353230	CCR7	G043H7	BV785	V15	T cell differentiation
BioLegend	359604	CD57	HNK-1	FITC	B2	T cell and NK cell differentiation
BioLegend	367148	CD14	63D3	Spark Blue 550	B3	Monocyte differentiation
BioLegend	368506	CD45	2D1	PerCP	B8	Leukocytes
BioLegend	303522	CD38	HIT2	PerCP-Cy5.5	B9	T cell, B cell, and NK cell activation and differentiation
BioLegend	303522	CD38	HIT2	PerCP-Cy5.5	B9	T cell, B cell, and NK cell activation and differentiation
BioLegend	300232	CD2	RPA-2.10	BV711	V13	NK cell differentiation
BD BioSciences	568933	CD15	7C3	BV750	V14	Neutrophils
BioLegend	353230	CCR7	G043H7	BV785	V15	T cell differentiation
BioLegend	359604	CD57	HNK-1	FITC	B2	T cell and NK cell differentiation

Company	Cat	Marker	Clone	Fluorophore	Mean Peak Channel	
BioLegend	367148	CD14	63D3	Spark Blue 550	B3	
BioLegend	368506	CD45	2D1	PerCP	B8	
BioLegend	303522	CD38	HIT2	PerCP-Cy5.5	B9	
Thermo	46-9959-42	TCRgd	B1.1	PerCP-eFluor 710	B10	
BioLegend	384704	CD125(IL-5Ra)	S20015E	PE	YG1	
Cytek	R7-20041	CD4	SK3	cFluor YG584	YG1	
BioLegend	351516	Siglec-9	K8	PE/Dazzle 594	YG3	
BioLegend	303406	CD33	WM53	PE-Cy5	YG5	
Cytek	R7-20009	CD19	HIB19	cFluor BYG710	YG7	
BioLegend	356108	CD25	M-A251	PE-Cy7	YG9	
BioLegend	356108	CD25	M-A251	PE-Cy7	YG9	T reg gating, T cell activation
BioLegend	347706	CD328 (Siglec-7)	S7.7	APC	R1	
BioLegend	310710	CCR3 (CD193)	5E8	Alexa Fluor 647	R2	Pan marker for granulocytes
BioLegend	304862	CD62L	DREG-56	Spark NIR 685	R3	
Cytek	R7-20089	CD56	5.1H11	cFluor R720	R4	NK cell differentiation
BioLegend	302816	CD27	0323	APC-Cy7	R7	T cell, B cell, and NK cell differentiation

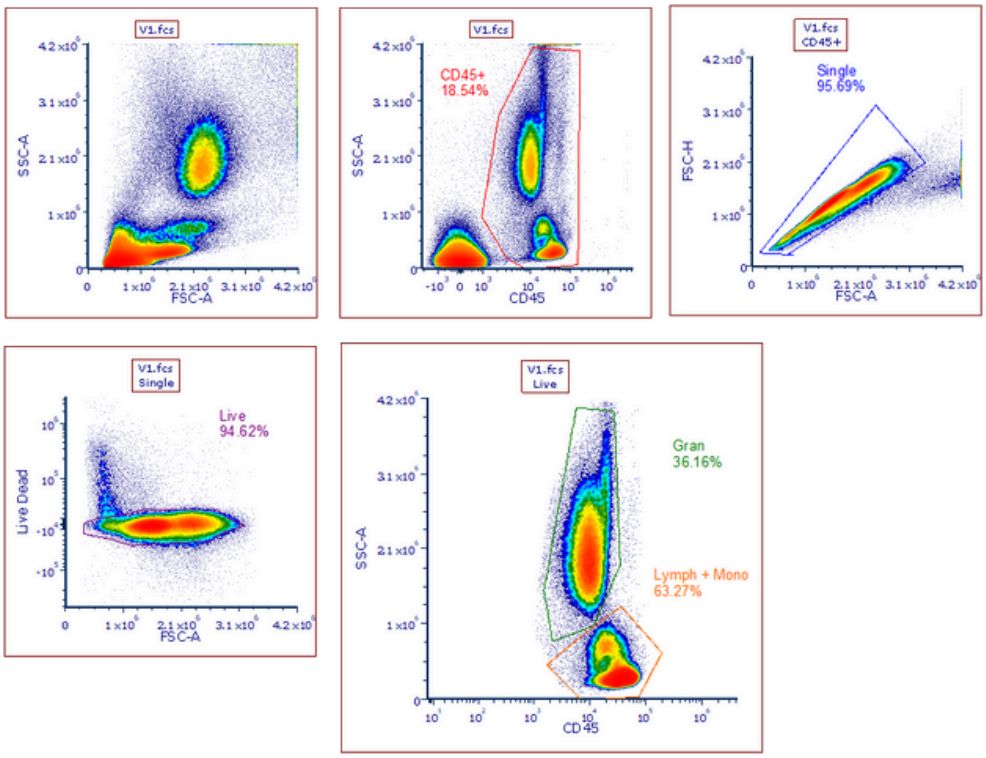
Table 2. Epitope Expression Analysis Antibody Panel

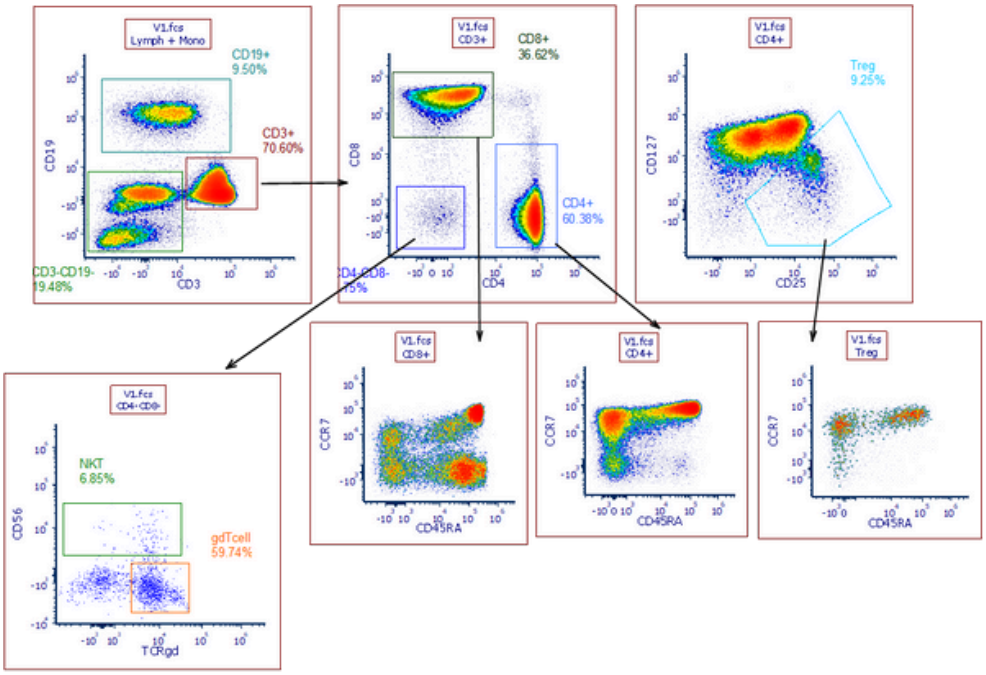
Antibody No.	Cat	Clone	Description	Flurophore
1	400908	HTK888	Armenian Hamster IgG Isotype control	PE
2	341504	6588-5	CCR10	PE
3	313508	C398.4A	CD278 (ICOS)	PE
4	400114	MOPC-21	Mouse IgG1, k isotype control	PE
5	352402	TRA-2-10	CD46	PE
6	300208	RPA-2.10	CD2	PE
7	316306	2M2	β2-microglobulin	PE
8	312204	HI10a	CD10	PE
9	328408	A8	CD100	PE
10	323006	W6D3	CD15	PE
11	352704	SHM14	CD156c (ADAM10	PE
12	302008	3G8	CD16	PE
13	328806	KPL-1	CD162	PE
14	352004	1A5	CD170 (Siglec-5)	PE
15	323806	SE5A5	CD172a/b	PE
16	346106	LMM741	CD114	PE
17	301208	HI111	CD11a	PE
18	301306	ICRF44	CD11b	PE
19	352804	UV4	CD126 (IL6Rα)	PE
20	301704	WM15	CD13	PE

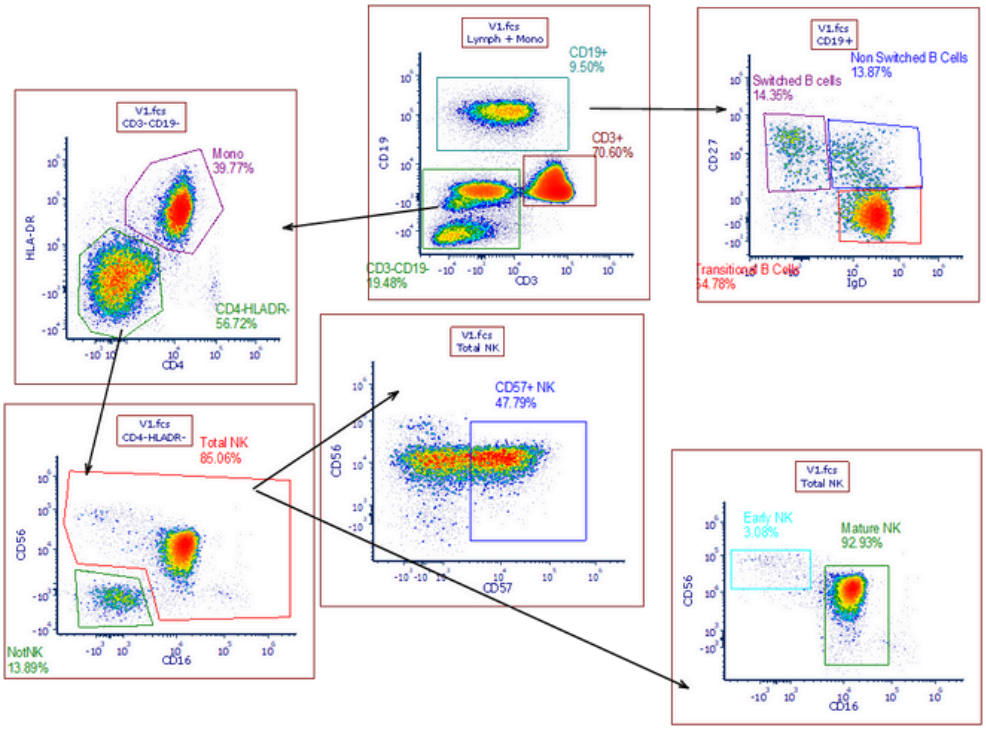
Antibody No.	Cat	Clone	Description	Flurophore
21	347404	HSL96	CD179a	PE
22	302108	TS1/18	CD18	PE
23	320706	5E8/CXCR2	CD182 (CXCR2)	PE
24	353706	G025H7	CD183	PE
25	302208	HIB19	CD19	PE
26	362904	5F10B29	CD191	PE
27	359412	L291H4	CD194	PE
28	311504	5F4	CD137L (4-1BB Ligand)	PE
29	367104	63D3	CD14	PE
30	344104	M80	CD141	PE
31	313808	H44	CD218a (IL-18Rα)	PE
32	329508	C1.7	CD244 (2B4)	PE
33	356104	M-A251	CD25	PE
34	356406	M-T271	CD27	PE
35	342704	BT3.1	CD277	PE
36	303004	TS2/16	CD29	PE
37	303706	HIP8	CD41	PE
38	303906	HIP1	CD42b	PE
39	304008	HI30	CD45	PE
40	300608	UCHT2	CD5	PE
41	311308	JS11	CD55	PE
42	362508	5.1H11	CD56	PE
43	330905	TS2/9	CD58	PE
44	341704	LNH-94	CD298	PE
45	300408	UCHT1	CD3	PE
46	303106	WM59	CD31	PE
47	320806	1D11	CD314 (NKG2D)	PE
48	348406	RS38E	CD317	PE
49	303404	WM53	CD33	PE
50	343506	581	CD34	PE
51	336406	VI-PL2	CD61	PE
52	304806	DREG-56	CD62L	PE
53	304906	AK4	CD62P (P-Selectin)	PE
54	353004	H5C6	CD63	PE
55	344706	SK1	CD8	PE
56	349506	5A6	CD81	PE
57	342104	ASL-24	CD82	PE
58	336906	VIM5	CD87	PE
59	333406	E11	CD35	PE
60	314906	TREM-26	CD354 (TREM-1)	PE
61	355306	9E8	CD367 (CLEC4A)	PE
62	303506	HIT2	CD38	PE
63	300508	RPA-T4	CD4	PE
64	312106	HI9a	CD9	PE
65	336108	VIMD2	CD93	PE

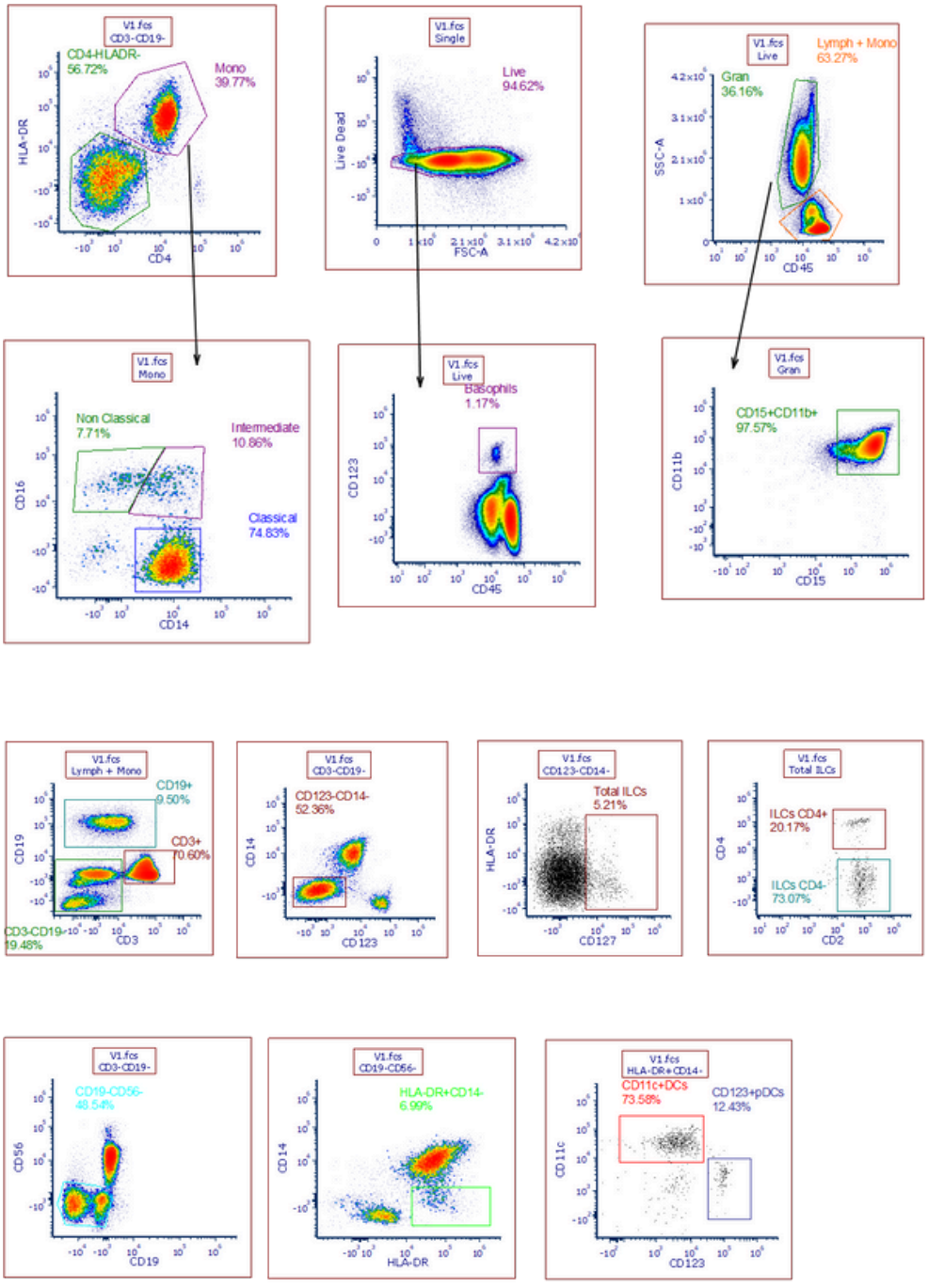
Antibody No.	Cat	Clone	Description	Flurophore
66	305608	DX2	CD95	PE
67	336308	VIM3b	CD97	PE
68	371306	3B2/TA8	CD99	PE
69	358204	CG4	GPR56	PE
70	342604	3D12		PE
71	367608	590H11G1E3	MERTK	PE
72	347703	S7.7	Siglec-7	PE
73	347104	7C9	Siglec-9	PE
74	400214	MOPC-173	Mouse IgG2a, k isotype control	PE
75	328506	CBR-IC2/2	CD102	PE
76	306506	12G5	CD184 (CXCR4)	PE
77	353204	G043H7	CD197 (CCR7)	PE
78	372304	W15177A	CD217	PE
79	311106	ML5	CD24	PE
80	348606	UIC2	CD243	PE
81	309708	TL2.1	CD282 (TLR2)	PE
82	336206	5-271	CD36	PE
83	355404	15E2	CD369 (Dectin-1/CLEC7A)	PE
84	353604	50C1	CD371 (CLEC12A)	PE
85	304206	UCHL1	CD45RO	PE
86	343106	CD7-6B7	CD7	PE
87	326008	CD84.1.21	CD84	PE
88	344304	S5/1	CD88	PE
89	311406	W6/32	HLA-A,B,C	PE
90	307606	L243	HLA-DR	PE
91	400314	MPC-11	Mouse IgG2b, k Isotype control	PE
92	302306	2H7	CD20	PE
93	363504	S-HCL-1	CD22	PE
94	304108	HI100	CD45RA	PE
95	310204	MEM-55	CD45RB	PE
96	400508	TRK2758	Rat IgG2a, k isotype control	PE
97	313612	GoH3	CD49f	PE
98	321204	FIB504	Integrin b7	PE
99	400636	RTK4530	Rat IgG2b, k isotype control	PE
100	341604	2A9-1	CX3CR1	PE

2.3 Suppl. Gating Strategy for the Delineation of Immune Cell Subpopulations in Human Whole Blood.

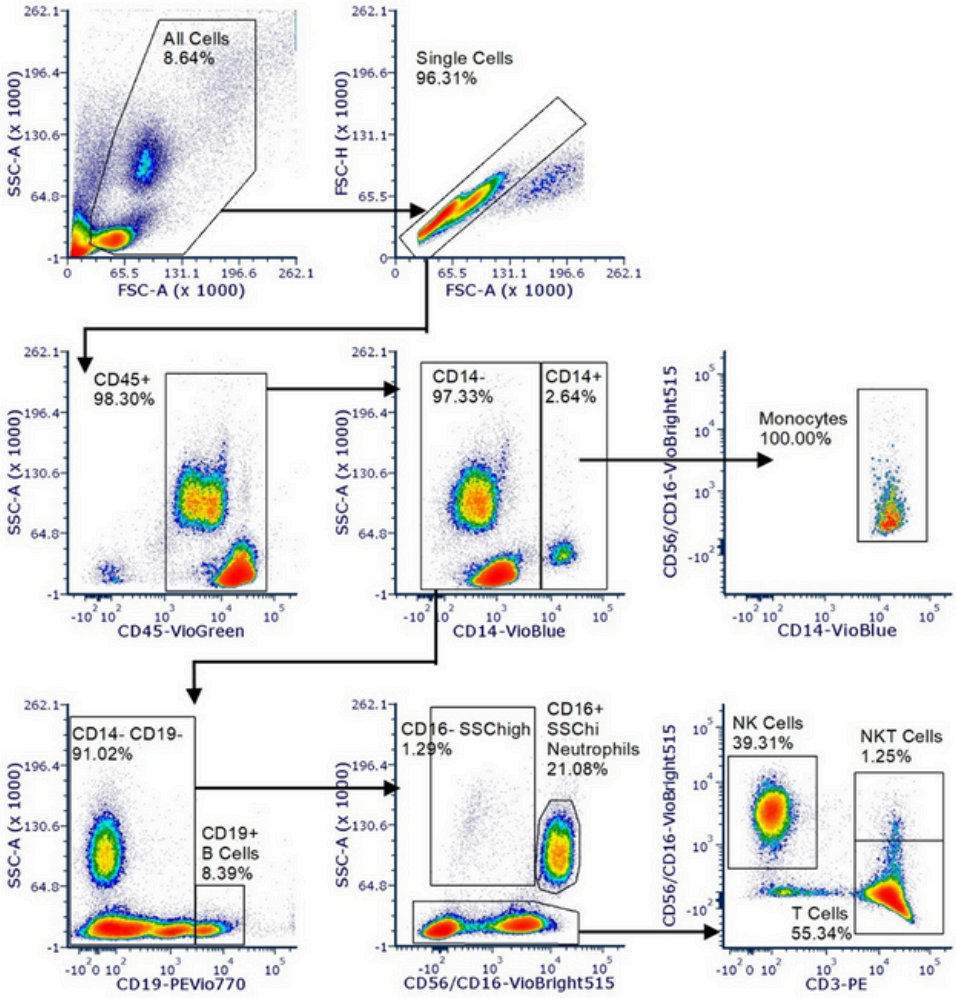



V1.fcs
Lymph + Mono
CD19+
9.50%
CD3+
70.60%
CD19
CD3
CD3-CD19-
19.48%
V1.fcs
CD3+
CD8+
36.62%
CD8
CD4
CD4+
60.38%
V1.fcs
CD4+
Treg
9.25%
CD127
CD25
V1.fcs
CD4-CD8-
NKT
6.85%
gdTcell
59.74%
CD56
TCRgd
V1.fcs
CD8+
CCR7
CD45RA
V1.fcs
CD4+
CCR7
CD45RA
V1.fcs
Treg
CCR7
CD45RA


V1.fcs
Lymph + Mono
CD19+
9.50%
CD3+
70.60%
CD19
CD3
CD3-CD19-
19.48%
V1.fcs
CD19+
Non Switched B Cells
13.87%
Switched B cells
14.36%
CD27
IgD
V1.fcs
CD3-CD19-
Mono
39.77%
HLA-DR
CD4
CD4-HLADR-
56.72%
V1.fcs
Total NK
CD57+ NK
47.79%
CD56
CD57
V1.fcs
CD4-HLADR-
Total NK
85.06%
CD56
CD16
NotNK
13.89%
V1.fcs
Total NK
Early NK
3.08%
Mature NK
92.93%
CD56
CD16


V1.fcs
CD3-CD19-
CD4-HLADR-
56.72%
Mono
39.77%
HLA-DR
CD4
V1.fcs
Single
Live
94.62%
Live Dead
FSC-A
V1.fcs
Live
Lymph + Mono
63.27%
Gran
36.16%
SSC-A
CD45
V1.fcs
Mono
Non Classical
7.71%
Intermediate
10.86%
Classical
74.83%
CD16
CD14
V1.fcs
Live
Basophils
1.17%
CD123
CD45
V1.fcs
Gran
CD15+CD11b+
97.57%
CD11b
CD15
V1.fcs
Lymph + Mono
CD19+
9.50%
CD3+
70.60%
CD19
CD3-CD19-
19.48%
CD3
V1.fcs
CD3-CD19-
CD123-CD14-
52.36%
CD14
CD123
V1.fcs
CD123-CD14-
Total ILCs
5.21%
HLA-DR
CD127
V1.fcs
Total ILCs
ILCs CD4+
20.17%
ILCs CD4-
73.07%
CD4
CD2
V1.fcs
CD3-CD19-
CD19-CD56-
48.54%
CD56
CD19
V1.fcs
CD19-CD56-
HLA-DR+CD14-
6.99%
CD14
HLA-DR
V1.fcs
HLA-DR+CD14-
CD11c+DCs
73.58%
CD123+pDCs
12.43%
CD11c
CD123

2.4 Suppl. Gating Strategy for the Delineation of Immune Cell Subpopulations in Human Whole Blood.



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6.0 ACKNOWLEDGEMENTS

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