
Cytek Biosciences

Cytek® cFluor® Human B Cell Monitoring Kit

Validation Report

This report describes the validation testing of the Cytek® cFluor® Human B Cell Monitoring Kit. This kit is a 13-color flow cytometry assay for the evaluation of human B cell lineage subsets in blood.

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1 Objective

To validate the performance of the 13-color B cell panel for the analysis of human B cell lineage subsets, as well as CD4⁺ and CD8⁺ T cells in blood.

2 Panel Design

The Cytek® cFluor® Human B Cell Monitoring Kit was designed to identify the following CD19⁺ B cell lineage subsets found in blood: naïve B cells (CD20⁺CD27⁻ IgD⁺), IgG class switched memory B cells (CD20⁺CD27⁺IgG⁺), unswitched memory B cells (CD20⁺CD27⁺ IgD⁺ and/or IgM⁺), and plasmablasts/plasma cells (CD20⁻ CD27⁺ CD38⁺⁺). Additionally, the kit identifies CD4⁺ and CD8⁺ subsets of CD3⁺ T cells. The tables below list the antibodies in the kit and describes the cell populations identified by the kit. Markers for CD14 and CD15 are for excluding monocytes and granulocytes from the lymphocyte gate.

Antibody list for Cytek® cFluor® Human B Cell Monitoring Kit

Blue			Red		
Specificity	Clone	Fluorochrome	Specificity	Clone	Fluorochrome
IgM	CH2	cFluor® B515	CD3	SK7	cFluor® R659
CD4	SK3	cFluor® B532	IgG	4A11	cFluor® R668
CD15	HI98	cFluor® B548	CD8	SK1	cFluor® R685
CD38	HB7	cFluor® BYG575	CD20	2H7	cFluor® R720
CD27	O323	cFluor® BYG610	CD45	2D1	cFluor® R780
CD14	MEM-15	cFluor® BYG667			
IgD	IgD26	cFluor® BYG710			
CD19	SJ25C1	cFluor® BYG781			

Populations identified in Cytek® cFluor® Human B Cell Monitoring Kit

B cell lineage subsets	Phenotype
Naïve	CD19+/CD20+/CD27-/IgD+
Unswitched Memory	CD19+/CD20+/CD27+/IgD+/IgM+
Switched Memory	CD19+/CD20+/CD27+/IgD-/IgG+
Plasmablasts/Plasma cells	CD19+/CD20-/CD38++/CD27+
T cell subsets	Phenotype
CD4+ (helper)	CD3+/CD4+
CD8+ (cytotoxic)	CD3+/CD8+
Myeloid populations	Phenotype
Monocytes	CD14+
Granulocytes	CD15+

3 Materials and Methods

3.1 Sample Type

Whole blood samples from healthy donors were tested. Whole blood collected in K₂EDTA (EDTA), heparin, and Cyto-Chex® BCT tubes were from Stanford Blood Center, AllCells or StemCell.

3.2 Reagents

- Cytek® cFluor® Human B Cell Monitoring Kit, Cytek Biosciences, R7-40008
- Cytek® FSP™ CompBeads, B7-10011
- Tonbo™ RBC Lysis Buffer (10X), TNB-4300-L100
- PBS, pH 7.4, Corning 21-040-CM
- Stain Buffer (BSA), BD Biosciences, 554657
- Tonbo™ Fixation Buffer, TNB-8222-L100

3.3 Instrument and Software

- Cytek® Aurora (5 laser) and Northern Lights™ (3 laser) systems
- SpectroFlo® software version 3.0.3

3.4 Methods

The Cytek® B cell panel was evaluated for:

1. Identifying B cell lineage subsets in blood collected in EDTA, heparin, and Cyto-Chex® tubes.
2. Determining the length of time following collection that blood in EDTA, heparin, and Cyto-Chex® tubes must be stained in order to preserve cell population patterns and percentages. (Age of Blood)
3. Determining the length of time following staining and fixation that samples must be acquired on a cytometer in order to retain cell population patterns and percentages. (Age of Stain)
4. Repeatability of the assay in generating the same results. (Precision)
5. Determining the length of time that the antibody cocktail should be stable for when stored at 2-8°C. (Cocktail Stability)

Testing and analysis methods:

Test Method	Analysis Method
Identifying B cell subsets in blood collected in EDTA, heparin, and Cyto-Chex® tubes Tested blood from the same donor collected in EDTA, heparin, and Cyto-Chex® tubes. Sample size: 3 donors, replicates of 3	Identified B and T cell populations and compared the patterns among all the samples.
Age of Blood (AOB) - Blood from the same donor collected in EDTA, heparin, and Cyto-Chex®* tubes were stained at <6 and 24h post-collection (24h blood were stored at room temperature) Age of Stain (AOS) - Stained samples were acquired at <6 and 24h post-staining (24h stained samples were stored at 2-8°C in the dark) Sample size: 3 donors, replicates of 3	Aged samples were compared to their respective Timepoint 0 (<6h AOB, <6h AOS) using the relative % difference in the %populations.
Precision 2 operators ran 10 donor samples in triplicates over 4 days. Sample size: 10 donors collected in heparin tubes, replicates of 3	Calculated the variation (%CV) in %populations of triplicates for each donor. The %CV of the 10 donors were averaged for each population.
Cocktail Stability Cocktails stored for 1, 2, 3, and 4 weeks at 2-8°C were compared to fresh. Sample size: 3 donors collected in heparin tubes	Aged cocktails were compared to a fresh cocktail using the relative % differences in the %populations and MFIs.

*Cyto-Chex® tubes were also tested at 48h AOB with 2 of the donors (48h blood was stored at room temperature)

Identifying B cell subsets in blood collected in EDTA, heparin, and Cyto-Chex® tubes

Blood from the same donor collected in EDTA, heparin, and Cyto-Chex® tubes were processed and stained within 6 hours from collection and acquired on a cytometer within 2 hours after staining. For making triplicates, RBC lysed samples were divided into 3 tubes and then stained. Three donors were used to identify populations and compare population patterns among the 3 tube types. Also, the variation (%CV) in the %populations among the 3 tube types per donor were determined.

Age of Blood (AOB) and Age of Stain (AOS)

AOB and AOS testing was combined and performed on 3 donors on separate days. Fresh whole blood collected in EDTA, heparin, and Cyto-Chex® tubes were stained at <6 and 24 hours post collection. Cyto-Chex® tubes were additionally tested at 48h post collection using 2 of the donors. Stained and fixed samples were analyzed on a cytometer at <6 and 24 hours post staining. The table below summarizes the testing schedule. For making triplicates, RBC lysed samples were divided into 3 tubes and then stained.

AOB/AOS Testing Schedule:

AOB	AOS	Staining			Acquisition			
		Day 0	Day 1	Day 2	Day 0	Day 1	Day 2	Day 3
<6h	<6h	X			X			
	24h					X		
24h	<6h		X			X		
	24h						X	
48h (CytoChex® tubes)	<6h			X			X	
	24h							X

Precision

Precision testing used 10 donors that were collected in heparin tubes. The blood was processed and stained within 26 hours from collection and acquired on a cytometer within 2 hours after staining. Each donor sample was processed in triplicates by an operator, whereby the whole blood was divided into 3 tubes and processed individually. The 10 donor samples were processed and acquired by 2 operators over 4 days. The table below summarizes the testing schedule.

Precision Testing Schedule:

Test Day	1			2		3			4	
Operator	1	1	2	1	2	1	1	2	1	2
Donor	1	2	3	4	5	6	7	8	9	10

Cocktail Stability

To make the cocktails, 5 µL of each antibody were mixed together per test. Cocktails were stored for 1, 2, 3, and 4 weeks at 2-8°C. The aged cocktails were compared to a fresh made cocktail using whole blood of 3 donors collected in heparin tubes. The blood was processed and stained within 26 hours from collection and acquired on a cytometer within 2 hours after staining.

3.4.1 Sample processing and staining of whole blood (DOC-00491)

Bulk-lysing Whole Blood

1. Collect whole blood into EDTA, heparin, or Cyto-Chex® tubes
2. Prepare a fresh working reagent of 1X RBC Lysis Buffer by diluting 1:10 with deionized water.
3. Transfer 45 mL of room temperature 1X lysis solution into a 50 mL conical tube
4. Transfer 5 mL of well mixed whole blood to the tube containing 45 mL of 1X lysis solution
5. Close and tighten the cap, mix gently by inverting or by placing the tube on a tube rocker for 5 minutes
6. Centrifuge at 400 x g, for 5 minutes

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7. Gently aspirate the supernatant without disturbing the pellet
8. Vortex gently
9. Add 50 mL of room temperature 1X lysis solution to the pellet, mix well
10. Repeat steps (5)-(7)
11. For heparin tubes* proceed to step 14. For EDTA and Cyto-Chex® tubes, vortex to resuspend the pellet and add 50 mL 1X PBS, mix well
12. Centrifuge at 400 x g, for 5 minutes
13. Gently aspirate the supernatant without disturbing the pellet
14. Resuspend in 1 mL of Stain Buffer

** Blood collected in heparin has significant loss of cells when washing after lysis due to difficulty in spinning the cells down into a pellet. It is recommended to proceed to re-resuspend in 1 mL stain buffer for staining following lysis.*

Single-Color Reference Controls

NOTE: Beads or cells can be used for Single-color Reference Controls.

1. Label a 12 x 75 mm test tube for each Single Stain Reference Control
2. For unstained tube add 50 µL of lysed cells
3. Add 50 µL of lysed cells or one drop of Cytek® FSP™ CompBeads to each Single-color Stain Reference Control tube
4. Add 5 µL of appropriate monoclonal antibody
5. Vortex thoroughly
6. Incubate for 20 minutes at room temperature, protected from light
7. Vortex and add 3 mL of Stain Buffer
8. Centrifuge at 400 x g, 5 minutes at room temperature
9. Decant or aspirate the supernatant
10. Repeat steps (7) to (9) for Cytek® FSP™ CompBeads, then resuspend beads in 200 µL Stain Buffer
11. Vortex and resuspend cells in 200 µL 1% paraformaldehyde
12. Acquire at medium flow rate within 6 hours post staining.

Multicolor Samples

1. Label a 12 x 75 mm test tube for each Multicolor sample
2. Prepare antibody cocktail according to the number of Multicolor samples. Add 5 µL per test of each antibody.
3. Add 200 µL of RBC lysed cells to Multicolor Sample tubes
4. Add 65 µL of the antibody cocktail prepared in step (2)
5. Vortex thoroughly
6. Incubate for 20 minutes at room temperature, protected from light
7. Vortex and add 3 mL of Stain Buffer

8. Centrifuge at 400 x g, 5 minutes at room temperature
9. Decant or aspirate the supernatant
10. Vortex thoroughly
11. Resuspend in 400 µL 1% paraformaldehyde
12. Acquire at medium or high flow rate within 4 hours post staining.

3.4.2 Flow Cytometry Analysis (DOC-00458)

- Flow rate: Medium or High
- Stopping Criteria:
 - Reference controls: 5,000 Beads; for cells see B cell kit template or DOC-00458
 - Multicolor sample: 1,000,000 events from "Cells" gate
- Threshold: FSC 350,000
- Autofluorescence Extraction:
 - No, for 2, 3, 4 laser instruments
 - Yes, for 5 laser instruments

3.4.3 Gating Strategy

Reference for identifying B cell lineage subsets

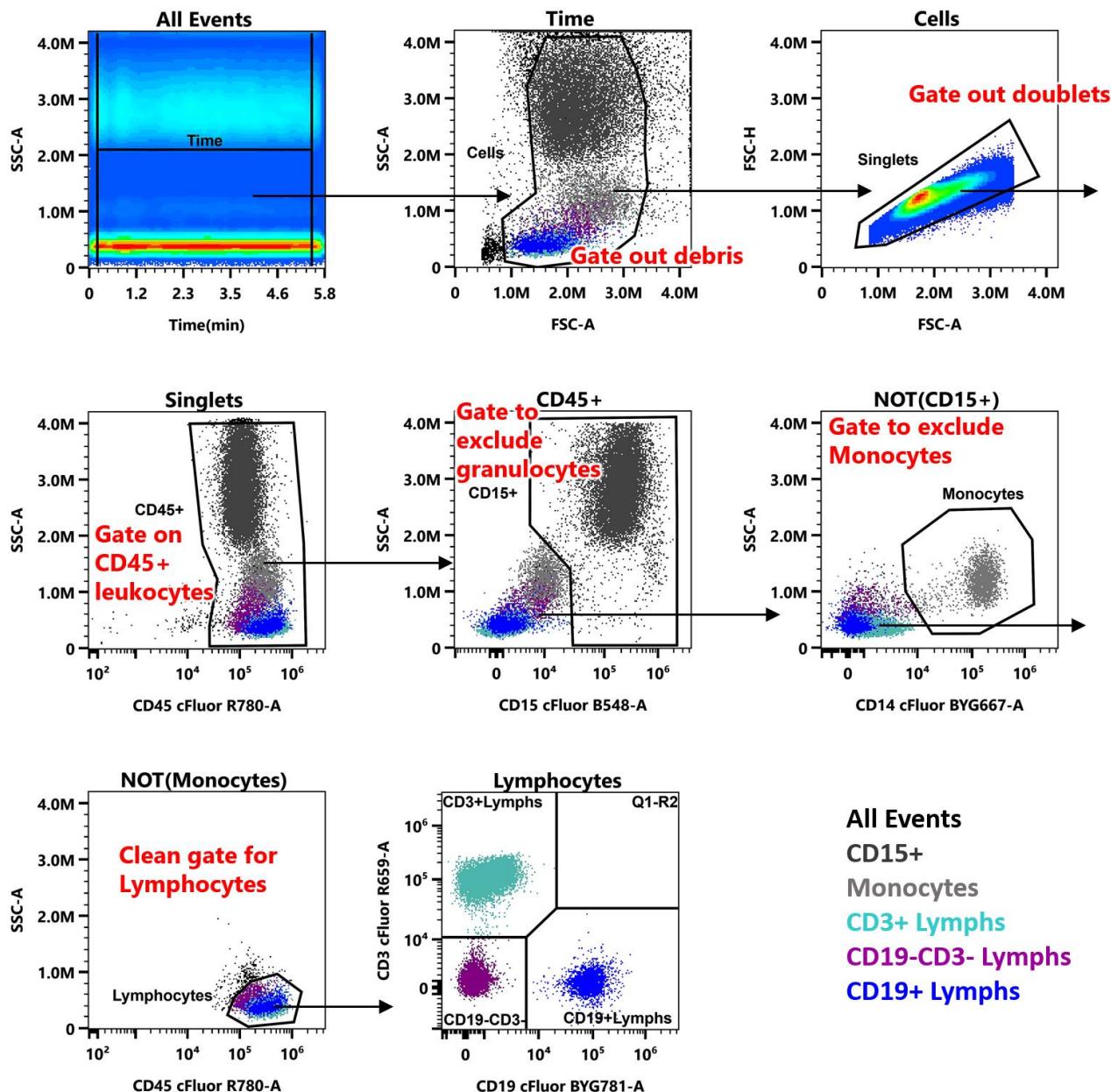
The Cytek® B cell panel design and gating strategy for identifying cell populations is based on Gatti et al.¹ This published B cell panel was used as a reference for the Cytek® panel to compare cell population patterns and percentages generated from blood. The finalized Cytek® panel had consistent results to the published panel.

¹ Gatti A, Buccisano F, Scupoli MT, Brando B. The ISCCA flow protocol for the monitoring of anti-CD20 therapies in autoimmune disorders. *Cytometry B Clin Cytom.* 2021 Mar;100(2):194-205. doi: 10.1002/cyto.b.21930. Epub 2020 Jun 29. PMID: 32598578.

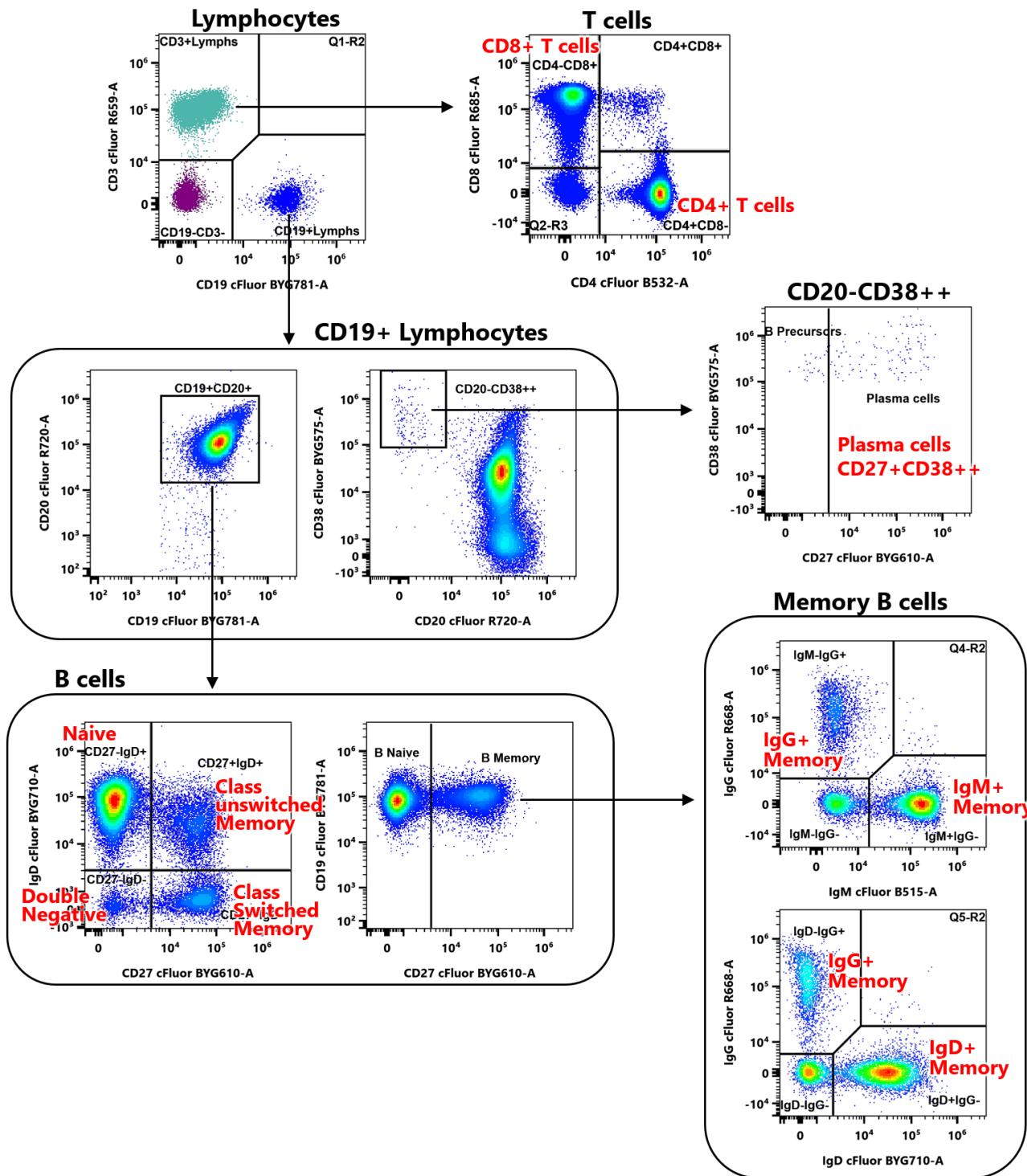
Gating strategy and logic:

Data shown was generated from normal whole blood collected in heparin.

A clean Lymphocyte population is achieved via gates of time, total cells, singlets, CD45⁺ leukocytes, not CD15⁺ granulocytes and not CD14⁺ monocytes gates.

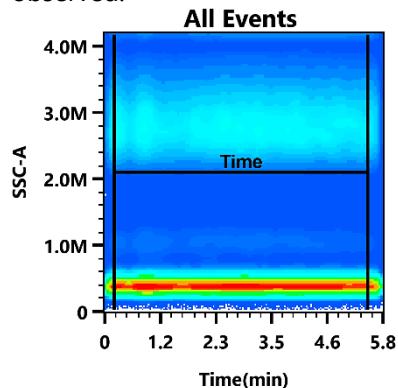


From the lymphocyte population, the B cell lineage subsets are distinguished, as well as T cell subsets.

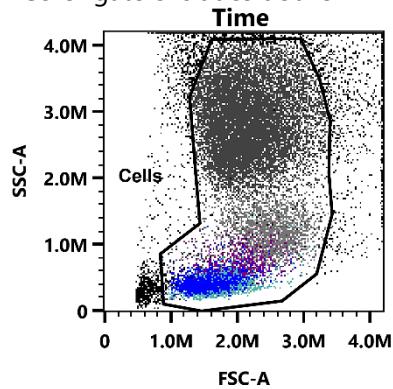


Parameters and gating for each plot:

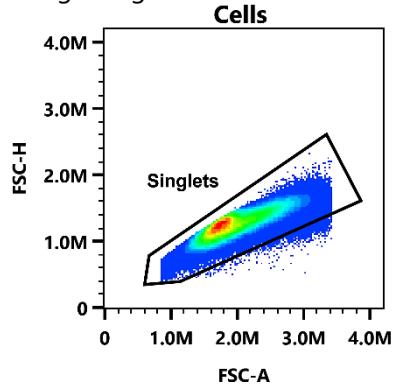
1. All Events: Time(min) vs SSC-A
 "Time" gate excludes any events at the start or end of the run where fluidics instability is observed.



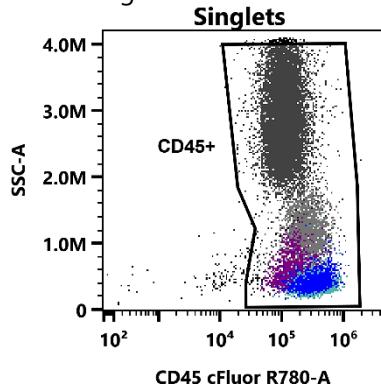
2. Time: FSC-A vs SSC-A
 "Cells" gate excludes debris



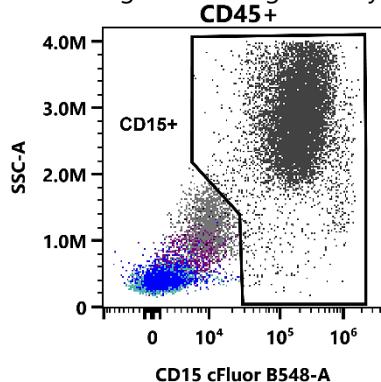
3. Cells: FSC-A vs FSC-H
 "Singlets" gate excludes doublets



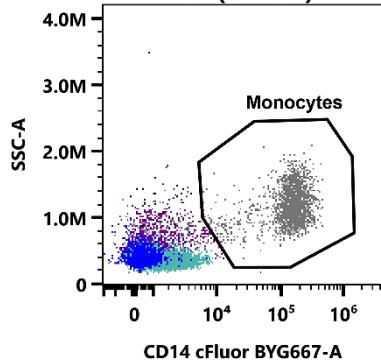
4. Singlets: CD45 cFluor® R780-A vs SSC-A
 "CD45+" gate selects CD45+ leukocytes



5. CD45+: CD15 cFluor® B548-A vs SSC-A
 "CD15+" gate selects granulocytes for exclusion

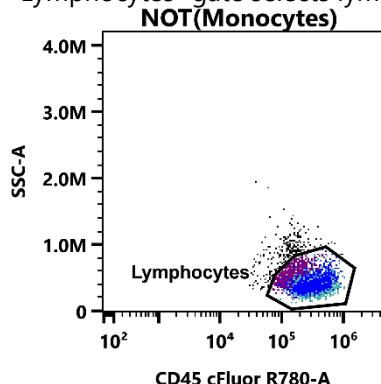


6. NOT(CD15+): CD14 cFluor® BYG667-A vs SSC-A
 "Monocytes" gate selects monocytes for exclusion



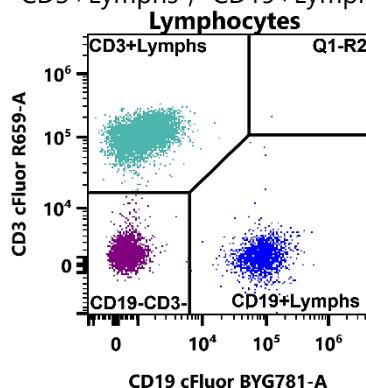
7. NOT(Monocytes): CD45 cFluor® R780-A vs SSC-A

"Lymphocytes" gate selects lymphocytes



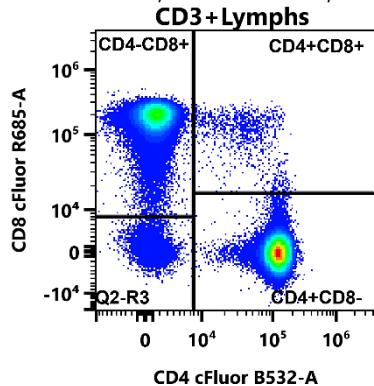
8. Lymphocytes: CD19 cFluor® BYG781-A vs CD3 cFluor® R659-A

"CD3+Lymphs", "CD19+Lymphs", "CD19-CD3-" gates distinguish B and T cells

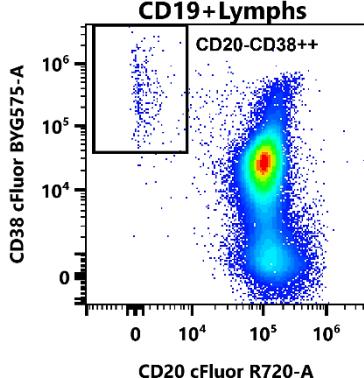


9. CD3+Lymphs: CD4 cFluor® B532-A vs CD8 cFluor® R685-A

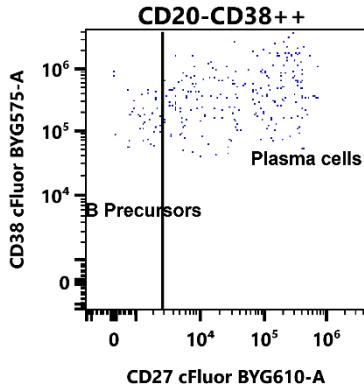
"CD4-CD8+", "CD4+CD8+", "CD4+CD8-" gates distinguish CD8⁺ and CD4⁺ T cells



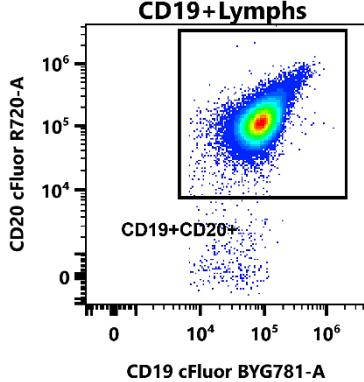
10. CD19+Lymphs: CD20 cFluor® R720-A vs CD38 cFluor® BYG575-A
 "CD20-CD38++" gate identifies Plasma cells and B precursors



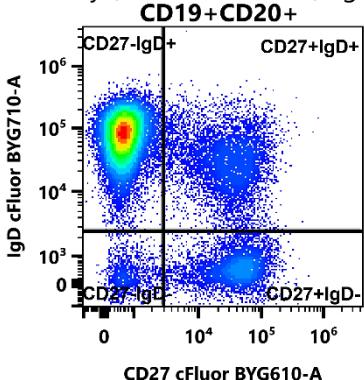
11. CD20-CD38++: CD27 cFluor® BYG610-A vs CD38 cFluor® BYG575-A
 "B Precursors" and "Plasma cells" are distinguished



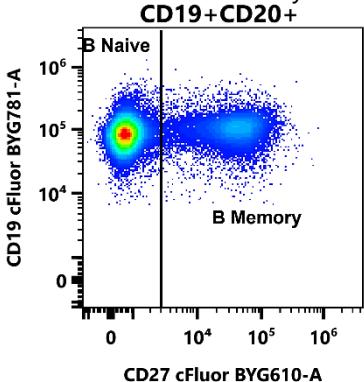
12. CD19+Lymphs: CD19 cFluor® BYG781-A vs CD20 cFluor® R720-A
 "CD19+CD20+" gate selects for B cells



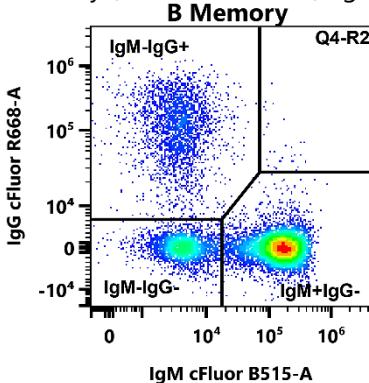
13. CD19+CD20+: CD27 cFluor® BYG610-A vs IgD cFluor® BYG710-A
 "CD27-IgD+", "CD27+IgD+", "CD27+IgD-", "CD27-IgD-" gates distinguish IgD⁺ naïve, IgD⁺ memory (class unswitched), IgD⁻ memory (class switched), double negative



14. CD19+CD20+: CD27 cFluor® BYG610-A vs CD19 cFluor® BYG781-A
 "B Naïve" and "B Memory" cells are distinguished

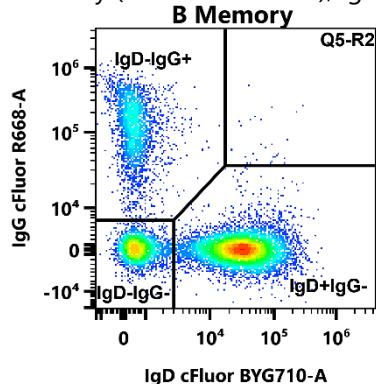


15. B Memory: IgM cFluor® B515-A vs IgG cFluor® R668-A
 "IgM-IgG+", "IgM+IgG-", "IgM-IgG-" gates distinguish IgG⁺ memory (class switched), IgM⁺ memory (class unswitched), IgM⁻IgG⁻ memory (IgA⁺, IgE⁺, or IgD⁺)



16. B Memory: IgD cFluor® BYG710-A vs IgG cFluor® R668-A

"IgD-IgG+", "IgD+IgG-", "IgD-IgG-" gates distinguish IgG⁺ memory (class switched), IgD⁺ memory (class unswitched), IgD⁻IgG⁻ memory (IgA⁺, IgE⁺, or IgM⁺)



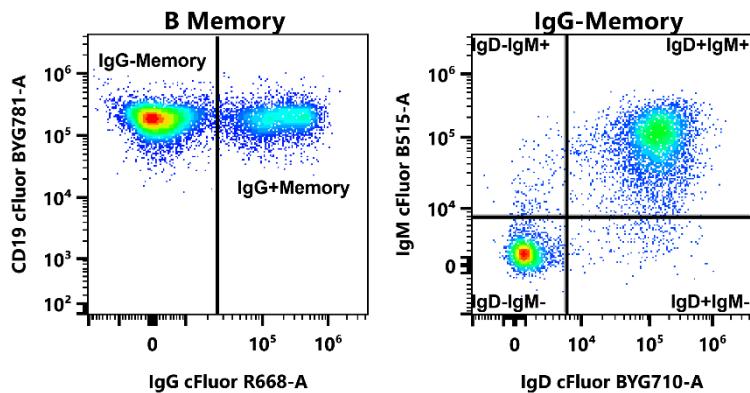
17. Additional gating for class switched and unswitched B Memory cells

B Memory: IgG cFluor® R668-A vs CD19 cFluor® BYG781-A

"IgG-" and "IgG+" gates distinguish IgG⁻ memory and IgG⁺ memory (IgG class switched)

IgG-Memory: IgD cFluor® BYG710-A vs IgM cFluor® B515-A

"IgD-IgM+", "IgD+IgM+", "IgD+IgM-", and "IgD-IgM-" gates distinguish IgD⁻IgM⁺ memory, IgD⁺IgM⁺ unswitched memory, IgD⁻IgM⁻ memory, and IgD⁺IgM⁻ memory (IgA⁺, IgE⁺)



4 Acceptance Criteria

Acceptance criteria for each test method:

Test and Analysis Method	Cell Population Relative %Difference or %CV
Identifying B cell subsets in blood collected in EDTA, heparin, and Cyto-Chex® tubes Compare population patterns generated from each collection tube <i>Analysis: Identification of populations</i>	CD3+ T cells of Lymphocytes ≤15% CD4+ T cells of Lymphocytes ≤15% CD8+ T cells of Lymphocytes ≤20% CD19+ B cells of Lymphocytes ≤20% CD20-CD27+CD38++ Plasma cells of CD19+ B cells ≤30% CD19+CD20+ B cells of Lymphocytes ≤20% CD19+CD27- B Naïve of Lymphocytes ≤25% CD19+CD27+ B Memory of CD19+CD20+ B cells ≤25% IgG+IgM- B Memory of CD19+CD20+ B cells ≤30% IgG-IgM+ B memory of CD19+CD20+ B cells ≤30% IgD+IgG- B memory of CD19+CD20+ B cells ≤30% IgD-IgG+ B memory of CD19+CD20+ B cells ≤30% CD27- IgD- Double Negative of CD19+CD20+ B cells ≤30% CD27+ IgD- B Memory of CD19+CD20+ B cells ≤30% CD27- IgD+ Naïve B of CD19+CD20+ B cells ≤30% CD27+ IgD+ B Memory of CD19+CD20+ B cells ≤30%
AOB/AOS Aged samples vs Time 0 <i>Analysis: Relative %Difference in %populations</i>	
Precision %CV in %populations of triplicates <i>Analysis: Mean %CV of 10 donors</i>	
Cocktail Stability Aged cocktails vs fresh cocktail <i>Analysis: Relative %Difference in %populations and MFI</i>	

5 Documentation and Archiving

Raw data and unmixed data were recorded on Aurora and Northern Lights™ instruments.

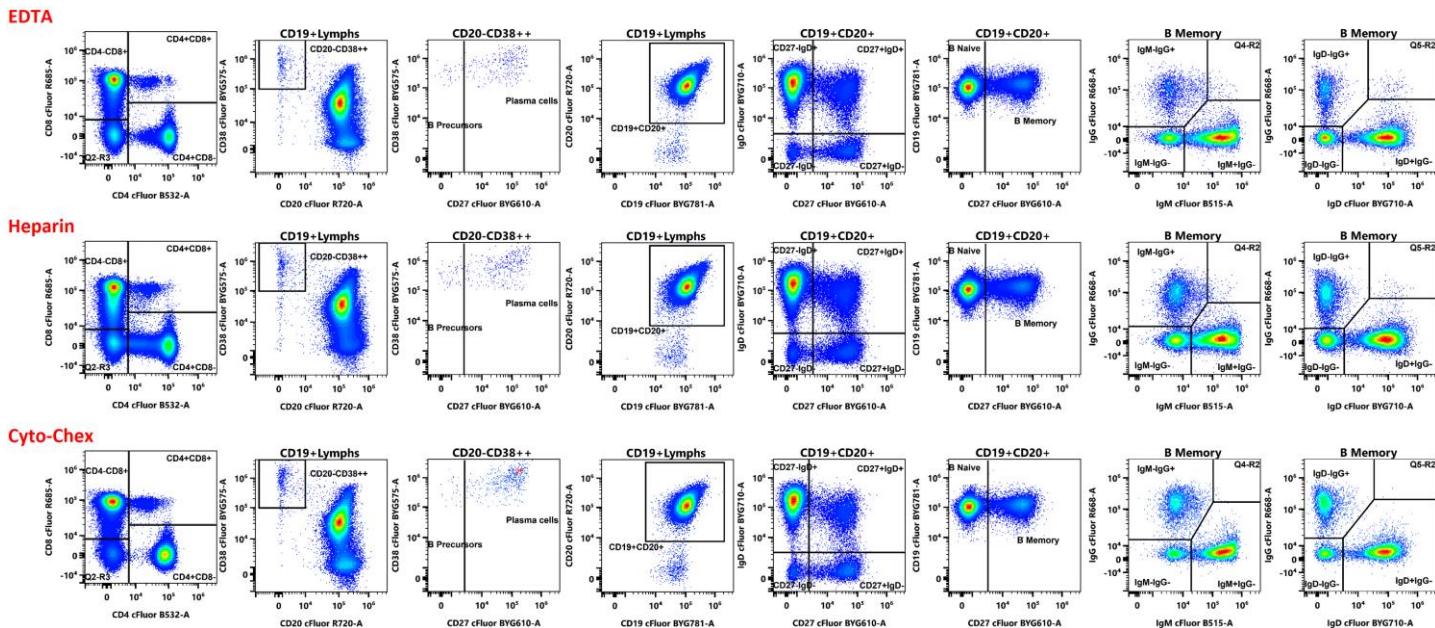
6 Responsibilities

The validation experiments were performed and reported by Heather Miller (Senior Scientist) and Kevin Tran (Scientist I). Data was reviewed and approved by Jun Deng (Director of Clinical Applications).

7 Results

7.1 Identifying cell subsets in blood collected in EDTA, heparin, and Cyto-Chex® tubes

The Cytek® cFluor® Human B Cell Monitoring Kit identified all the target cell populations with similar patterns in blood collected in EDTA, heparin, and Cyto-Chex® tubes. The plots below are from one donor and is representative of 3 donors tested.



The %CV in %populations were used to determine the variation among the 3 tube types. Table 8.1.1 shows the %populations for 3 donors in each collection tube. Table 8.1.2 shows the %CV in the 3 tube types for each donor and the average of the %CVs for the 3 donors. From the averages, only the plasma cell population failed the acceptance criteria (see Section 4), having a %CV >30%. This was due to the Cyto-Chex® tubes having a higher %population than EDTA and heparin.

Table 8.1.1: % Populations for 3 donors collected in EDTA, heparin, and Cyto-Chex® tubes

Population	Donor 1			Donor 2			Donor 3		
	EDTA	Heparin	CytoChex	EDTA	Heparin	CytoChex	EDTA	Heparin	CytoChex
CD3+Lymphs % Lymphs	52.00	55.70	65.87	73.08	71.79	71.65	57.80	62.29	73.40
CD4-CD8+ % Lymphs	17.88	17.71	24.76	29.45	29.06	28.68	30.54	33.30	36.08
CD4+CD8- % Lymphs	28.18	32.00	37.05	37.99	37.21	37.75	15.16	18.74	28.77
CD19+Lymphs % Lymphs	16.54	12.80	10.74	9.21	11.39	10.71	26.59	25.07	14.95
CD19+CD20+ % Lymphs	16.38	12.58	10.49	8.43	10.49	9.56	26.45	24.93	14.71
B Naive % Lymphs	6.91	5.57	4.37	6.05	7.57	7.09	20.02	18.88	10.47
Plasma cells % CD19+Lymphs	0.72	1.28	1.92	8.15	7.46	10.11	0.41	0.42	1.33
CD27-IgD+ % CD19+CD20+	36.59	37.40	35.45	69.35	70.05	71.62	73.02	72.27	66.15
CD27+IgD+ % CD19+CD20+	27.44	25.49	27.15	15.87	15.48	13.54	15.17	14.08	16.44
CD27-IgD- % CD19+CD20+	6.10	6.99	6.85	1.99	1.74	2.47	3.25	3.91	4.30
CD27+IgD- % CD19+CD20+	29.86	30.13	30.55	12.79	12.72	12.37	8.56	9.74	13.11
B Memory % CD19+CD20+	57.81	55.72	58.31	28.15	27.83	26.07	24.28	24.29	28.84
IgM-IgG+ % CD19+CD20+	19.73	19.77	19.62	8.50	8.29	8.13	3.72	4.91	8.42
IgM+IgG- % CD19+CD20+	27.64	25.14	28.58	14.87	14.79	14.31	15.49	14.04	15.50
IgD-IgG+ % CD19+CD20+	19.40	19.49	20.39	8.58	8.17	8.17	3.72	5.07	8.45
IgD+IgG- % CD19+CD20+	27.72	25.41	28.25	15.65	15.15	14.12	15.35	14.22	15.72

Table 8.1.2: %CV in %Populations for 3 donors collected in EDTA, heparin, and Cyto-Chex® tubes

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Population	Donor 1	Donor 2	Donor 3	Average of %CV	Acceptance Criteria
CD3+Lymphs % Lymphs	12.41	1.09	12.45	8.65	≤15%
CD4-CD8+ % Lymphs	20.00	1.31	8.32	9.88	≤20%
CD4+CD8- % Lymphs	13.72	1.07	33.79	16.19	≤15%
CD19+Lymphs % Lymphs	21.99	10.67	28.51	20.39	≤20%
CD19+CD20+ % Lymphs	22.70	10.89	29.00	20.86	≤20%
B Naive % Lymphs	22.56	11.23	31.69	21.83	≤25%
Plasma cells %	46.23	16.07	73.45	45.25	≤30%
CD27-IgD- % CD19+CD20+	2.68	1.65	5.35	3.23	≤30%
CD27+IgD+ %	3.94	8.35	7.74	6.68	≤30%
CD27-IgD- % CD19+CD20+	7.17	17.87	13.86	12.97	≤30%
CD27+IgD- % CD19+CD20+	1.16	1.79	22.58	8.51	≤30%
B Memory % CD19+CD20+	2.40	4.09	10.19	5.56	≤25%
IgM-IgG+ % CD19+CD20+	0.38	2.24	43.04	15.22	≤30%
IgM+IgG- % CD19+CD20+	6.55	2.09	5.60	4.75	≤30%
IgD-IgG+ % CD19+CD20+	2.76	2.86	42.43	16.02	≤30%
IgD+IgG- % CD19+CD20+	5.58	5.20	5.18	5.32	≤30%

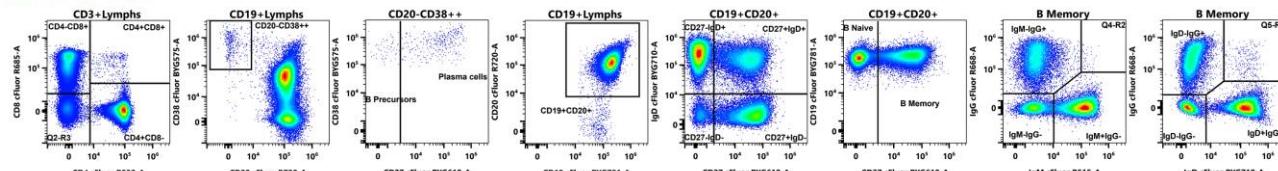
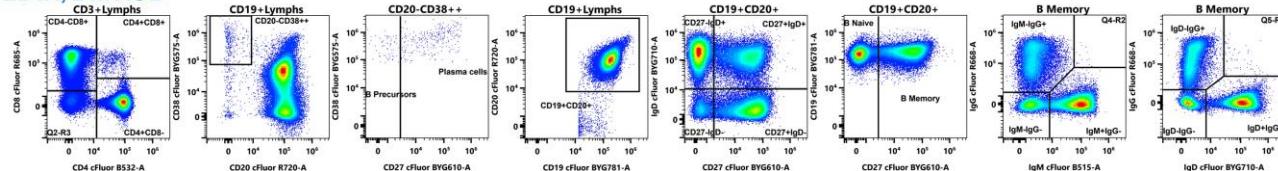
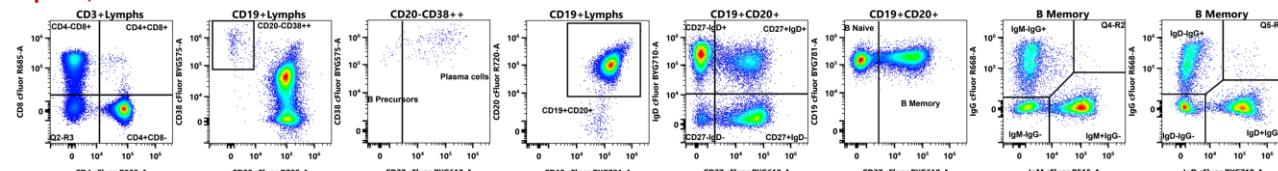
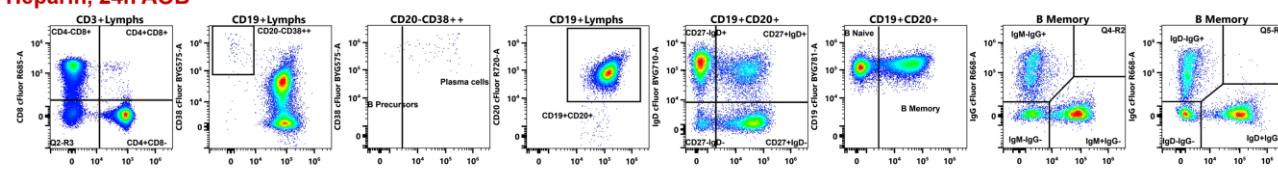
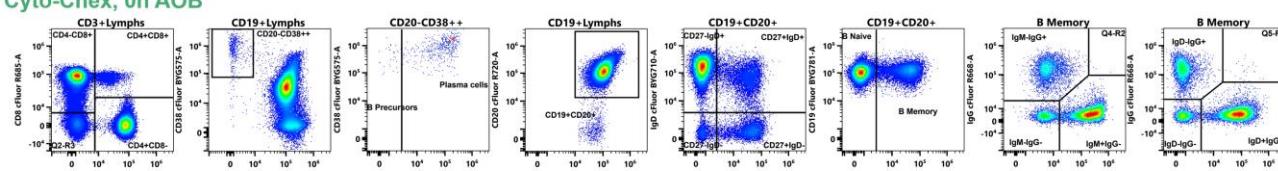
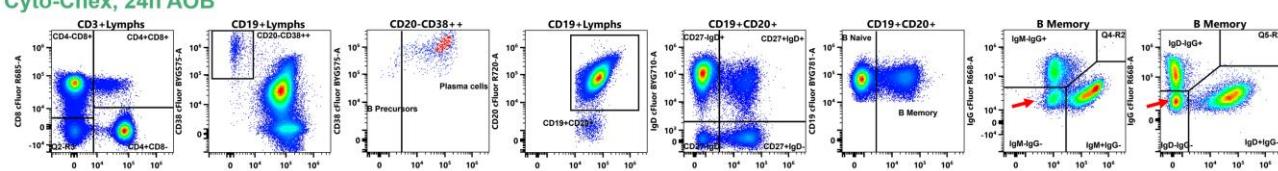
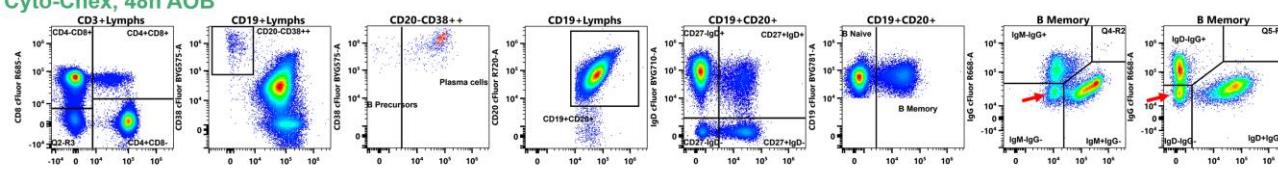
7.2 AOB/AOS

AOB Results

The relative %difference in %populations between 24h AOB and 0h AOB was averaged from the 3 donors for each collection tube. From the averages, the 24h AOB EDTA samples passed all criteria, except for CD8⁺T cells, which failed slightly, and plasma cells, which had ~50% decrease and >30% decrease for each donor (Table 8.2.1). The 24h AOB heparin samples passed all criteria, except for the plasma cells, which had ~60% decrease and >50% decrease for each donor (Table 8.2.2). Therefore, using fresh blood is recommended for EDTA and heparin tubes. The 24h AOB Cyto-Chex® tube samples passed all the criteria (Table 8.2.3).

Additionally, the 2 donors tested at 48h AOB passed all criteria except for the CD27⁻IgD⁻ population (Table 8.2.4), which increased in %population.

The plots below are representative of 3 donors for each collection tube. The population patterns were similar from 0h to 24h AOB for all tubes, except for Cyto-Chex® tubes, where the IgG⁻ B memory cells were shifted higher on the IgG cFluor® R668 axis (red arrows).

EDTA, 0h AOB

EDTA, 24h AOB

Heparin, 0h AOB

Heparin, 24h AOB

Cyto-Chek, 0h AOB

Cyto-Chek, 24h AOB

Cyto-Chek, 48h AOB


The tables below show the relative %difference of the aged samples to their respective time 0 and the averages of the 3 donors.

Table 8.2.1- EDTA Samples: %Difference 24h AOB vs 0h AOB

Populations	Acceptance Criteria	Donor			Average
		1	2	3	
CD3+Lymphs % Lymphs	≤15%	19.84	2.46	10.39	10.90
CD4-CD8+ % Lymphs	≤20%	52.06	-1.96	21.85	23.98
CD4+CD8- % Lymphs	≤15%	7.09	3.22	5.01	5.11
CD19+Lymphs % Lymphs	≤20%	-20.16	1.92	-11.46	-9.90
CD19+CD20+ % Lymphs	≤20%	-19.87	8.86	-11.24	-7.41
B Naive % Lymphs	≤25%	-23.02	10.41	-18.12	-10.24
Plasma cells % CD19+Lymphs	≤30%	-47.26	-76.38	-37.11	-53.58
CD27-IgD+ % CD19+CD20+	≤30%	-7.17	0.82	-9.41	-5.25
CD27+IgD+ % CD19+CD20+	≤30%	-0.87	-3.70	12.63	2.69
CD27-IgD- % CD19+CD20+	≤30%	17.27	5.70	38.77	20.58
CD27+IgD- % CD19+CD20+	≤30%	6.07	-0.73	43.20	16.18
B Memory % CD19+CD20+	≤25%	2.85	-3.47	23.71	7.70
IgM-IgG+ % CD19+CD20+	≤30%	3.01	-9.49	83.42	25.65
IgM+IgG- % CD19+CD20+	≤30%	1.33	-0.96	9.96	3.44
IgD-IgG+ % CD19+CD20+	≤30%	4.50	-5.98	83.86	27.46
IgD+IgG- % CD19+CD20+	≤30%	2.00	-4.24	14.68	4.15

Table 8.2.2- Heparin: %Difference 24h AOB vs 0h AOB

Populations	Acceptance Criteria	Donor			Average
		1	2	3	
CD3+Lymphs % Lymphs	≤15%	6.53	1.11	1.20	2.94
CD4-CD8+ % Lymphs	≤20%	33.57	-1.38	5.96	12.71
CD4+CD8- % Lymphs	≤15%	-6.13	0.38	-6.30	-4.02
CD19+Lymphs % Lymphs	≤20%	-16.18	-4.62	-1.26	-7.35
CD19+CD20+ % Lymphs	≤20%	-15.37	0.83	-0.96	-5.17
B Naive % Lymphs	≤25%	-11.55	1.76	-5.19	-4.99
Plasma cells % CD19+Lymphs	≤30%	-65.52	-69.89	-59.24	-64.88
CD27-IgD+ % CD19+CD20+	≤30%	2.90	0.43	-5.23	-0.64
CD27+IgD+ % CD19+CD20+	≤30%	-1.14	-4.05	6.27	0.36
CD27-IgD- % CD19+CD20+	≤30%	13.02	14.91	16.21	14.72
CD27+IgD- % CD19+CD20+	≤30%	-5.66	0.60	23.23	6.06
B Memory % CD19+CD20+	≤25%	-3.54	-2.30	10.96	1.71
IgM-IgG+ % CD19+CD20+	≤30%	-10.76	-7.52	26.29	2.67
IgM+IgG- % CD19+CD20+	≤30%	0.61	9.78	-0.33	3.35
IgD-IgG+ % CD19+CD20+	≤30%	-9.80	-3.55	32.41	6.35
IgD+IgG- % CD19+CD20+	≤30%	-0.34	-3.39	4.24	0.17

Table 8.2.3- Cyto-Chex®: %Difference 24h AOB vs 0h AOB

Populations	Acceptance Criteria	Donor			Average
		1	2	3	
CD3+Lymphs % Lymphs	≤15%	0.32	0.02	1.52	0.62
CD4-CD8+ % Lymphs	≤20%	1.62	-2.95	-5.22	-2.19
CD4+CD8- % Lymphs	≤15%	-1.02	0.35	10.62	3.32
CD19+Lymphs % Lymphs	≤20%	1.77	-0.78	-0.91	0.03
CD19+CD20+ % Lymphs	≤20%	2.19	1.50	-1.41	0.76
B Naive % Lymphs	≤25%	11.59	5.69	0.64	5.97
Plasma cells % CD19+Lymphs	≤30%	-17.50	-17.86	20.14	-5.07
CD27-IgD+ % CD19+CD20+	≤30%	5.06	2.28	0.15	2.49
CD27+IgD+ % CD19+CD20+	≤30%	-12.60	-10.76	-15.45	-12.94
CD27-IgD- % CD19+CD20+	≤30%	31.40	25.64	27.70	28.25
CD27+IgD- % CD19+CD20+	≤30%	-1.72	-6.50	9.56	0.45
B Memory % CD19+CD20+	≤25%	-6.55	-12.65	-3.24	-7.48
IgM-IgG+ % CD19+CD20+	≤30%	-14.61	-14.80	2.18	-9.08
IgM+IgG- % CD19+CD20+	≤30%	-5.97	-15.94	-10.00	-10.64
IgD-IgG+ % CD19+CD20+	≤30%	-15.47	-12.69	7.69	-6.82
IgD+IgG- % CD19+CD20+	≤30%	-9.24	-18.86	-12.70	-13.60

Table 8.2.4- Cyto-Chex®: %Difference 48h AOB vs 0h AOB

Populations	Acceptance Criteria	Donor		Average
		2	3	
CD3+Lymphs % Lymphs	≤15%	-0.88	1.88	0.50
CD4-CD8+ % Lymphs	≤20%	-6.01	-2.46	-4.23
CD4+CD8- % Lymphs	≤15%	0.88	7.78	4.33
CD19+Lymphs % Lymphs	≤20%	5.10	6.74	5.92
CD19+CD20+ % Lymphs	≤20%	8.89	6.62	7.75
B Naive % Lymphs	≤25%	17.90	8.31	13.11
Plasma cells % CD19+Lymphs	≤30%	-27.32	-3.65	-15.48
CD27-IgD+ % CD19+CD20+	≤30%	6.15	0.97	3.56
CD27+IgD+ % CD19+CD20+	≤30%	-26.52	-17.72	-22.12
CD27-IgD- % CD19+CD20+	≤30%	50.20	31.42	40.81
CD27+IgD- % CD19+CD20+	≤30%	-16.60	6.99	-4.81
B Memory % CD19+CD20+	≤25%	-24.92	-3.09	-14.00
IgM-IgG+ % CD19+CD20+	≤30%	-24.72	1.03	-11.85
IgM+IgG- % CD19+CD20+	≤30%	-26.12	-6.71	-16.41
IgD-IgG+ % CD19+CD20+	≤30%	32.03	3.59	14.22
IgD+IgG- % CD19+CD20+	≤30%	32.48	-11.62	-22.05

AOS Results

The 3 collection tubes passed the AOS criteria for all timepoints, thus the 1% PFA fixation of samples and storage at 2-8°C in the dark maintains population patterns and %populations.

The tables below show the relative %difference of the aged samples to their respective time 0 and the averages of the 3 donors.

Table 8.2.5- EDTA: 0h AOB, %Difference 24h AOS vs 0h AOS

Populations	Acceptance Criteria	Donor			Average
		1	2	3	
CD3+Lymphs % Lymphs	≤15%	-0.96	1.25	1.14	0.48
CD4-CD8+ % Lymphs	≤20%	0.69	2.80	1.81	1.77
CD4+CD8- % Lymphs	≤15%	-1.37	0.54	0.75	-0.03
CD19+Lymphs % Lymphs	≤20%	-0.32	1.23	0.21	0.37
CD19+CD20+ % Lymphs	≤20%	-0.31	3.32	0.26	1.09
B Naive % Lymphs	≤25%	-1.01	1.76	1.12	0.62
Plasma cells % CD19+Lymphs	≤30%	-2.18	-22.32	-10.23	-11.58
CD27-IgD+ % CD19+CD20+	≤30%	-0.23	-1.83	-0.25	-0.77
CD27+IgD+ % CD19+CD20+	≤30%	2.40	8.49	2.31	4.40
CD27-IgD- % CD19+CD20+	≤30%	-3.88	-10.22	-3.49	-5.86
CD27+IgD- % CD19+CD20+	≤30%	-1.12	0.96	-0.70	-0.28
B Memory % CD19+CD20+	≤25%	0.55	3.98	-3.57	0.32
IgM-IgG+ % CD19+CD20+	≤30%	-1.67	2.20	-0.90	-0.12
IgM+IgG- % CD19+CD20+	≤30%	-1.34	5.29	-4.67	-0.24
IgD-IgG+ % CD19+CD20+	≤30%	-2.30	2.06	0.81	0.19
IgD+IgG- % CD19+CD20+	≤30%	2.39	6.31	-4.06	1.55

Table 8.2.6- Heparin: 0h AOB, %Difference 24h AOS vs 0h AOS

Populations	Acceptance Criteria	Donor			Average
		1	2	3	
CD3+Lymphs % Lymphs	≤15%	-3.15	0.76	-0.31	-0.90
CD4-CD8+ % Lymphs	≤20%	-2.00	1.41	-2.80	-1.13
CD4+CD8- % Lymphs	≤15%	-2.82	0.73	-0.12	-0.74
CD19+Lymphs % Lymphs	≤20%	-1.59	-1.14	0.39	-0.78
CD19+CD20+ % Lymphs	≤20%	-1.54	1.81	0.44	0.24
B Naive % Lymphs	≤25%	-1.80	-0.13	1.04	-0.30
Plasma cells % CD19+Lymphs	≤30%	1.94	-36.20	-5.99	-13.42
CD27-IgD+ % CD19+CD20+	≤30%	-0.32	-2.17	-0.50	-1.00
CD27+IgD+ % CD19+CD20+	≤30%	1.92	11.58	3.95	5.82
CD27-IgD- % CD19+CD20+	≤30%	0.10	-10.13	-2.47	-4.17
CD27+IgD- % CD19+CD20+	≤30%	-1.27	-0.73	-0.99	-1.00
B Memory % CD19+CD20+	≤25%	0.19	4.97	-5.75	-0.20
IgM-IgG+ % CD19+CD20+	≤30%	-1.42	1.77	-7.27	-2.31
IgM+IgG- % CD19+CD20+	≤30%	-2.21	8.52	-6.10	0.07
IgD-IgG+ % CD19+CD20+	≤30%	-1.95	1.18	-6.05	-2.27
IgD+IgG- % CD19+CD20+	≤30%	0.68	9.50	-6.35	1.28

Table 8.2.7- Cyto-Chex®: 0h AOB, %Difference 24h AOS vs 0h AOS

Populations	Acceptance Criteria	Donor			Average
		1	2	3	
CD3+Lymphs % Lymphs	≤15%	-0.31	1.02	-0.44	0.09
CD4-CD8+ % Lymphs	≤20%	0.78	2.16	-1.23	0.57
CD4+CD8- % Lymphs	≤15%	-0.67	0.55	-0.74	-0.29
CD19+Lymphs % Lymphs	≤20%	1.55	-0.16	0.45	0.61
CD19+CD20+ % Lymphs	≤20%	1.59	0.59	0.39	0.86
B Naive % Lymphs	≤25%	-1.45	-1.64	1.21	-0.63
Plasma cells % CD19+Lymphs	≤30%	-1.66	-6.15	4.23	-1.19
CD27-IgD+ % CD19+CD20+	≤30%	-2.09	-1.89	0.21	-1.26
CD27+IgD+ % CD19+CD20+	≤30%	2.15	9.46	-3.53	2.69
CD27-IgD- % CD19+CD20+	≤30%	-5.45	-7.96	12.57	-0.28
CD27+IgD- % CD19+CD20+	≤30%	1.72	2.24	-0.76	1.07
B Memory % CD19+CD20+	≤25%	2.14	6.39	-3.64	1.63
IgM-IgG+ % CD19+CD20+	≤30%	2.09	3.73	-5.30	0.17
IgM+IgG- % CD19+CD20+	≤30%	0.08	8.97	-4.26	1.60
IgD-IgG+ % CD19+CD20+	≤30%	1.28	3.59	-4.38	0.16
IgD+IgG- % CD19+CD20+	≤30%	2.82	9.94	-5.60	2.39

Table 8.2.8- Cyto-Chex®: 24h AOB, %Difference 24h AOS vs 0h AOS

Populations	Acceptance Criteria	Donor			Average
		1	2	3	
CD3+Lymphs % Lymphs	≤15%	0.44	0.48	-0.45	0.16
CD4-CD8+ % Lymphs	≤20%	0.21	1.26	1.39	0.95
CD4+CD8- % Lymphs	≤15%	0.38	0.39	-1.25	-0.16
CD19+Lymphs % Lymphs	≤20%	0.40	0.78	-0.65	0.18
CD19+CD20+ % Lymphs	≤20%	1.15	1.06	-0.69	0.51
B Naive % Lymphs	≤25%	1.98	1.56	-2.28	0.42
Plasma cells % CD19+Lymphs	≤30%	-41.78	-2.52	-0.20	-14.83
CD27-IgD+ % CD19+CD20+	≤30%	1.12	0.40	-0.43	0.36
CD27+IgD+ % CD19+CD20+	≤30%	-1.05	-1.46	0.70	-0.61
CD27-IgD- % CD19+CD20+	≤30%	0.56	4.51	2.13	2.40
CD27+IgD- % CD19+CD20+	≤30%	-0.72	-2.19	0.51	-0.80
B Memory % CD19+CD20+	≤25%	-0.73	-1.65	3.83	0.48
IgM-IgG+ % CD19+CD20+	≤30%	1.07	-3.13	4.88	0.94
IgM+IgG- % CD19+CD20+	≤30%	-1.13	-2.99	5.74	0.54
IgD-IgG+ % CD19+CD20+	≤30%	0.66	-4.77	-0.04	-1.38
IgD+IgG- % CD19+CD20+	≤30%	-0.66	-1.40	4.69	0.88

Table 8.2.9- Cyto-Chex®: 48h AOB, %Difference 24h AOS vs 0h AOS

Populations	Acceptance Criteria	Donor		Average
		2	3	
CD3+Lymphs % Lymphs	≤15%	0.39	-1.70	-0.65
CD4-CD8+ % Lymphs	≤20%	0.96	-1.27	-0.15
CD4+CD8- % Lymphs	≤15%	0.42	-1.58	-0.58
CD19+Lymphs % Lymphs	≤20%	1.69	-1.80	-0.05
CD19+CD20+ % Lymphs	≤20%	1.57	-1.93	-0.18
B Naive % Lymphs	≤25%	0.92	-1.00	-0.04
Plasma cells % CD19+Lymphs	≤30%	2.26	10.05	6.15
CD27-IgD+ % CD19+CD20+	≤30%	-0.60	-0.13	-0.36
CD27+IgD+ % CD19+CD20+	≤30%	8.41	0.71	4.56
CD27-IgD- % CD19+CD20+	≤30%	-7.37	-2.60	-4.98
CD27+IgD- % CD19+CD20+	≤30%	-1.03	1.02	-0.01
B Memory % CD19+CD20+	≤25%	2.37	-5.19	-1.41
IgM-IgG+ % CD19+CD20+	≤30%	2.29	-1.41	0.44
IgM+IgG- % CD19+CD20+	≤30%	0.06	-7.22	-3.58
IgD-IgG+ % CD19+CD20+	≤30%	-0.54	-4.95	-2.75
IgD+IgG- % CD19+CD20+	≤30%	5.94	-6.64	-0.35

7.3 Precision

The assay proved to be highly repeatable, with the average %CV from 10 donors for all cell populations being considerably lower than the passing criteria specifications.

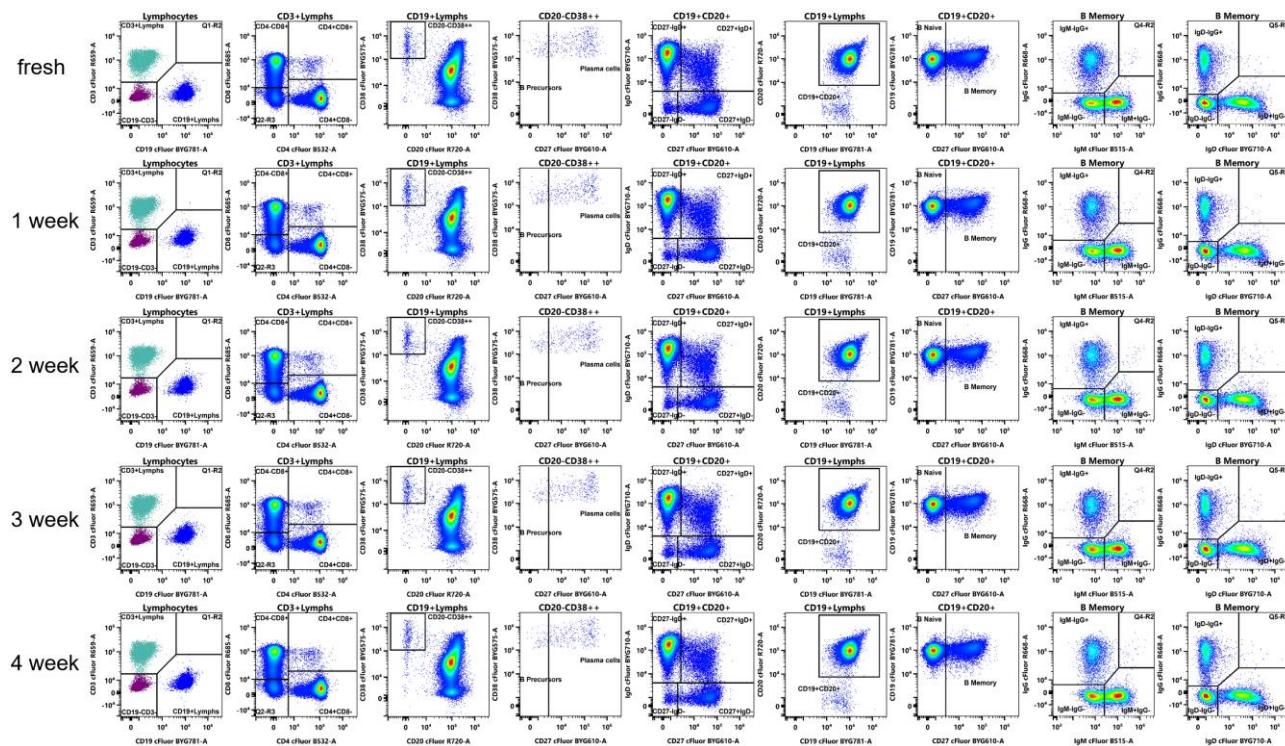
The table below shows the %CV of triplicates for each donor (D) for the indicated %populations and the averages of the %CVs of the 10 donors.

Populations	%CV for 3 Replicates										Avg %CV	Passing Criteria
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10		
CD3+Lymphs % Lymphs	0.42	0.50	2.47	0.97	0.65	1.68	0.92	0.97	0.68	0.65	0.99	≤15%
CD4-CD8+ % Lymphs	3.28	0.52	2.78	2.39	0.61	0.67	1.40	3.39	2.13	5.03	2.22	≤20%
CD4+CD8- % Lymphs	0.74	0.85	2.18	0.84	0.65	3.38	1.37	1.57	0.24	4.26	1.61	≤15%
CD19+Lymphs % Lymphs	2.06	6.27	4.11	3.92	4.23	8.07	2.83	18.18	3.59	12.17	6.54	≤20%
CD19+CD20+ % Lymphs	2.08	6.23	4.12	3.91	4.24	8.13	2.78	18.20	3.60	12.35	6.56	≤20%
B Naive % Lymphs	1.93	5.13	2.02	3.35	5.19	9.03	2.91	18.52	3.98	16.36	6.84	≤25%
Plasma cells % CD19+Lymphs	14.53	6.11	16.51	1.71	19.43	18.07	15.72	22.50	1.71	11.73	12.80	≤30%
CD27-IgD+ % CD19+CD20+	0.92	1.50	5.08	0.61	1.36	1.48	0.36	1.74	0.88	4.97	1.89	≤30%
CD27+IgD+ % CD19+CD20+	4.92	2.16	2.42	1.66	2.27	1.70	0.67	4.05	1.21	4.76	2.58	≤30%
CD27-IgD- % CD19+CD20+	4.11	2.82	5.50	0.75	6.65	1.35	1.79	10.71	4.45	5.18	4.33	≤30%
CD27+IgD- % CD19+CD20+	5.20	0.57	2.32	3.75	2.16	4.10	2.87	6.92	3.60	6.05	3.76	≤30%
B Memory % CD19+CD20+	5.05	1.45	1.95	2.08	1.92	2.72	0.97	4.97	2.36	5.06	2.85	≤25%
IgM-IgG+ % CD19+CD20+	5.54	2.18	1.35	2.84	0.37	4.72	2.02	7.07	2.33	6.57	3.50	≤30%

IgM+IgG- % CD19+CD20+	7.20	1.71	2.42	2.14	1.97	1.46	0.18	4.02	2.43	5.28	2.88	≤30%
IgD-IgG+ % CD19+CD20+	5.38	1.53	1.47	2.92	1.23	4.75	2.03	7.04	2.39	6.38	3.51	≤30%
IgD+IgG- % CD19+CD20+	5.51	2.11	2.39	1.64	2.12	1.75	0.81	4.16	1.90	4.76	2.72	≤30%

7.4 Cocktail Stability

The antibody cocktail passed all criteria for stability testing up to 4 weeks, which was the longest timepoint tested. The aged cocktails generated the same population patterns as the fresh cocktail. The below plots are representative of the 3 donors.



The tables below show the %populations and MFI values for the 3 donors stained with the fresh or 1-4 week cocktails. Also, the relative %difference in %populations and MFI of aged vs fresh cocktails are shown, along with the averages.

%Difference in %Populations, Aged Cocktails vs Fresh Cocktail

Population	CD3+Lymphs % Lymphs	CD4-CD8+ % Lymphs	CD4+CD8- % Lymphs	CD19+Lymphs % Lymphs	CD19+CD20+ % Lymphs	B Naive % Lymphs	Plasma cells % CD19+B cells	
Acceptance Criteria	≤15%	≤20%	≤15%	≤20%	≤20%	≤25%	≤30%	
Donor 1	Fresh	75.21	41.62	28.92	5.78	5.60	3.46	2.51
	1wk	74.02	41.24	27.99	5.84	5.65	3.50	2.46
	2wk	74.10	41.91	27.35	5.99	5.81	3.63	2.35
	3wk	74.46	40.21	29.55	5.94	5.77	3.62	2.43
	4wk	73.89	42.39	26.54	6.51	6.36	4.01	1.77
	%Diff 1wk	-1.58	-0.91	-3.22	1.04	0.89	1.16	-1.99
	%Diff 2wk	-1.48	0.70	-5.43	3.63	3.75	4.91	-6.22
	%Diff 3wk	-1.00	-3.39	2.18	2.77	3.04	4.62	-3.09
	%Diff 4wk	-1.76	1.85	-8.23	12.63	13.57	15.90	-29.42
Donor 2	Fresh	44.26	12.34	30.70	26.68	26.08	19.89	1.91
	1wk	43.62	12.44	29.92	28.20	27.57	21.10	1.82
	2wk	44.04	12.62	30.10	26.51	25.91	19.60	1.95
	3wk	44.21	13.12	29.78	24.99	24.36	18.14	2.18
	4wk	43.91	12.76	29.85	26.55	25.92	19.44	1.99
	%Diff 1wk	-1.45	0.81	-2.54	5.70	5.71	6.08	-4.37
	%Diff 2wk	-0.50	2.27	-1.95	-0.64	-0.65	-1.46	1.96
	%Diff 3wk	-0.11	6.32	-3.00	-6.33	-6.60	-8.80	14.47
	%Diff 4wk	-0.79	3.40	-2.77	-0.49	-0.61	-2.26	4.46
Donor 3	Fresh	60.74	25.78	29.09	17.73	17.57	13.43	0.59
	1wk	59.47	26.35	27.28	17.60	17.43	13.30	0.64
	2wk	59.83	26.62	27.20	17.59	17.42	13.28	0.65
	3wk	59.38	26.58	26.86	18.07	17.91	13.66	0.57
	4wk	59.13	27.42	25.67	17.46	17.30	13.19	0.61
	%Diff 1wk	-2.09	2.21	-6.22	-0.73	-0.80	-0.97	9.61
	%Diff 2wk	-1.50	3.26	-6.50	-0.79	-0.85	-1.12	11.13
	%Diff 3wk	-2.24	3.10	-7.67	1.92	1.94	1.71	-2.71
	%Diff 4wk	-2.65	6.36	-11.76	-1.52	-1.54	-1.79	4.14
Avg of Donors	%Diff 1wk	-1.71	0.70	-3.99	2.00	1.94	2.09	1.09
	%Diff 2wk	-1.16	2.07	-4.63	0.74	0.75	0.78	2.29
	%Diff 3wk	-1.12	2.01	-2.83	-0.55	-0.54	-0.82	2.89
	%Diff 4wk	-1.73	3.87	-7.58	3.54	3.81	3.95	-6.94

Population	CD27-IgD+ % CD19+CD20+	CD27+IgD+ % CD19+CD20+	CD27-IgD- % CD19+CD20+	CD27+IgD- % CD19+CD20+	B Memory % CD19+CD20+	IgM-IgG+ % CD19+CD20+	IgM+IgG- % CD19+CD20+	IgD-IgG+ % CD19+CD20+	IgD+IgG- % CD19+CD20+	
Acceptance Criteria	≤30%	≤30%	≤30%	≤30%	≤25%	≤30%	≤30%	≤30%	≤30%	
Donor 1	Fresh	60.56	27.35	0.94	11.15	38.19	2.17	27.16	2.23	27.56
	1wk	60.60	27.62	0.96	10.83	38.01	2.06	27.01	2.09	27.67
	2wk	60.84	27.45	1.03	10.68	37.63	2.10	26.99	2.15	27.52
	3wk	61.37	27.58	0.92	10.14	37.31	2.03	27.47	2.08	27.71
	4wk	61.94	27.13	0.79	10.14	36.90	1.83	26.95	1.84	27.22
	%Diff 1wk	0.07	0.99	2.13	-2.87	-0.47	-5.07	-0.55	-6.28	0.40
	%Diff 2wk	0.46	0.37	9.57	-4.22	-1.47	-3.23	-0.63	-3.59	-0.15
	%Diff 3wk	1.34	0.84	-2.13	-9.06	-2.30	-6.45	1.14	-6.73	0.54
	%Diff 4wk	2.28	-0.80	-15.96	-9.06	-3.38	-15.67	-0.77	-17.49	-1.23
Donor 2	Fresh	73.48	8.45	2.45	15.61	23.73	7.29	7.07	7.37	8.00
	1wk	73.64	8.49	2.53	15.34	23.47	7.56	6.66	7.60	7.90
	2wk	72.79	8.72	2.50	15.99	24.38	7.45	7.25	7.54	8.30
	3wk	71.36	9.05	2.74	16.85	25.52	8.03	7.44	8.07	8.58
	4wk	72.02	8.80	2.62	16.56	25.00	7.78	7.35	7.82	8.32
	%Diff 1wk	0.22	0.47	3.27	-1.73	-1.10	3.70	-5.80	3.12	-1.25
	%Diff 2wk	-0.94	3.20	2.04	2.43	2.74	2.19	2.55	2.31	3.75
	%Diff 3wk	-2.89	7.10	11.84	7.94	7.54	10.15	5.23	9.50	7.25
	%Diff 4wk	-1.99	4.14	6.94	6.09	5.35	6.72	3.96	6.11	4.00
Donor 3	Fresh	74.39	10.68	1.66	13.27	23.57	6.60	8.89	6.64	10.59
	1wk	74.35	11.10	1.57	12.97	23.70	6.25	9.53	6.30	10.99
	2wk	74.26	11.06	1.58	13.10	23.77	6.49	9.27	6.49	10.89
	3wk	74.16	11.22	1.69	12.94	23.73	6.25	9.44	6.28	11.08
	4wk	74.14	10.93	1.63	13.30	23.77	6.56	9.37	6.63	10.73
	%Diff 1wk	-0.05	3.93	-5.42	-2.26	0.55	-5.30	7.20	-5.12	3.78
	%Diff 2wk	-0.17	3.56	-4.82	-1.28	0.85	-1.67	4.27	-2.26	2.83
	%Diff 3wk	-0.31	5.06	1.81	-2.49	0.68	-5.30	6.19	-5.42	4.63
	%Diff 4wk	-0.34	2.34	-1.81	0.23	0.85	-0.61	5.40	-0.15	1.32
Avg of donors	%Diff 1wk	0.08	1.80	-0.01	-2.29	-0.34	-2.22	0.28	-2.76	0.98
	%Diff 2wk	-0.22	2.37	2.27	-1.02	0.71	-0.90	2.06	-1.18	2.15
	%Diff 3wk	-0.62	4.33	3.84	-1.20	1.97	-0.53	4.19	-0.88	4.14
	%Diff 4wk	-0.01	1.89	-3.61	-0.92	0.94	-3.18	2.86	-3.84	1.36

%Difference in MFI, Aged Cocktails vs Fresh Cocktail

Population	Lymphs CD45 R780	CD3+Lymphs CD3 R659	CD3+CD8+ CD8 R685	CD3+CD4+ CD4 B532	CD19+Lymphs CD19 BYG781
Acceptance Criteria	≤15%	≤15%	≤20%	≤15%	≤20%
Donor 1	Fresh	300,971	89,872	77,033	94,802
	1wk	296,797	83,927	78,585	87,497
	2wk	287,749	83,635	76,801	85,951
	3wk	290,996	84,719	78,315	84,513
	4wk	275,254	85,282	76,497	80,502
	%Diff 1wk	-1.39	-6.61	2.01	-7.71
	%Diff 2wk	-4.39	-6.94	-0.30	-9.34
	%Diff 3wk	-3.31	-5.73	1.66	-10.85
	%Diff 4wk	-8.54	-5.11	-0.70	-15.08
Donor 2	Fresh	265,145	102,417	91,548	102,293
	1wk	268,423	96,838	97,843	94,303
	2wk	253,675	96,947	96,795	96,190
	3wk	263,566	96,223	98,737	94,124
	4wk	248,455	98,251	94,947	88,686
	%Diff 1wk	1.24	-5.45	6.88	-7.81
	%Diff 2wk	-4.33	-5.34	5.73	-5.97
	%Diff 3wk	-0.60	-6.05	7.85	-7.99
	%Diff 4wk	-6.29	-4.07	3.71	-13.30
Donor 3	Fresh	297,329	101,243	68,744	97,555
	1wk	301,995	98,681	73,402	94,833
	2wk	280,581	95,277	70,218	92,802
	3wk	287,010	95,370	71,030	89,356
	4wk	273,326	97,360	70,364	97,657
	%Diff 1wk	1.57	-2.53	6.78	-2.79
	%Diff 2wk	-5.63	-5.89	2.14	-4.87
	%Diff 3wk	-3.47	-5.80	3.33	-8.40
	%Diff 4wk	-8.07	-3.84	2.36	0.10
Avg	%Diff 1wk	0.47	-4.86	5.22	-6.10
	%Diff 2wk	-4.78	-6.06	2.52	-6.72
	%Diff 3wk	-2.46	-5.86	4.28	-9.08
	%Diff 4wk	-7.64	-4.34	1.79	-9.43

	Population	CD19+CD20+ CD20 R720	B Memory CD27 BYG610	IgM+IgG- B Memory IgM B515	IgD-IgG+ B Memory IgG R668	IgD+IgG- B Memory IgD BYG710	Plasma cells CD38 BYG575
	Acceptance Criteria	≤20%	≤30%	≤30%	≤30%	≤30%	≤30%
Donor 1	Fresh	105,347	19,936	161,580	68,238	30,937	703,327
	1wk	108,115	20,082	154,413	63,186	31,769	658,674
	2wk	106,043	20,204	158,371	64,125	32,130	650,342
	3wk	109,079	20,597	154,786	65,506	31,347	646,271
	4wk	103,476	19,632	157,426	58,012	31,090	567,599
	%Diff 1wk	2.63	0.73	-4.44	-7.40	2.69	-6.35
	%Diff 2wk	0.66	1.34	-1.99	-6.03	3.86	-7.53
	%Diff 3wk	3.54	3.32	-4.20	-4.00	1.33	-8.11
	%Diff 4wk	-1.78	-1.52	-2.57	-14.99	0.49	-19.30
Donor 2	Fresh	84,761	40,793	108,629	72,827	55,972	570,414
	1wk	88,011	39,974	108,239	78,503	53,908	624,893
	2wk	85,700	40,878	109,987	76,988	53,091	651,445
	3wk	89,002	40,627	109,363	73,222	58,089	646,666
	4wk	82,787	40,122	106,569	74,042	55,957	604,084
	%Diff 1wk	3.83	-2.01	-0.36	7.79	-3.69	9.55
	%Diff 2wk	1.11	0.21	1.25	5.71	-5.15	14.21
	%Diff 3wk	5.00	-0.41	0.68	0.54	3.78	13.37
	%Diff 4wk	-2.33	-1.64	-1.90	1.67	-0.03	5.90
Donor 3	Fresh	89,664	28,405	77,789	77,883	36,461	387,480
	1wk	96,429	28,913	84,270	79,477	39,359	438,708
	2wk	92,401	29,118	82,572	77,969	39,650	454,251
	3wk	93,747	28,167	82,027	76,148	38,977	421,173
	4wk	88,968	28,002	84,228	73,518	38,411	429,404
	%Diff 1wk	7.54	1.79	8.33	2.05	7.95	13.22
	%Diff 2wk	3.05	2.51	6.15	0.11	8.75	17.23
	%Diff 3wk	4.55	-0.84	5.45	-2.23	6.90	8.70
	%Diff 4wk	-0.78	-1.42	8.28	-5.60	5.35	10.82
Avg	%Diff 1wk	4.67	0.17	1.18	0.81	2.32	5.47
	%Diff 2wk	1.61	1.35	1.80	-0.07	2.49	7.97
	%Diff 3wk	4.37	0.69	0.64	-1.90	4.00	4.65
	%Diff 4wk	-1.63	-1.53	1.27	-6.31	1.94	-0.86

8 Summary

The table below describes the testing results and specifications for the Cytek® cFluor® Human B Cell Monitoring Kit.

Test and Analysis Method	Results and Specifications
Identifying B cell populations in blood collected in EDTA, heparin, and Cyto-Chex® tubes Compared population patterns generated from each collection tube <i>Analysis: Identification of populations</i>	Passed: Samples collected in EDTA, heparin, and Cyto-Chex® tubes generated similar population patterns that identified the target B lineage subsets and T subsets
AOB/AOS Aged samples vs Time 0 <i>Analysis: Relative %Difference of %populations</i>	<ul style="list-style-type: none"> • EDTA and Heparin: At 24h AOB the plasma cells decreased by ~50% • Cyto-Chex®: Passed 24h AOB and 24h AOS At 48h AOB the CD27-IgD- B cells increased over 30% • Stained and 1% PFA fixed samples stored at 2-8°C in the dark for 24h maintained population patterns, %populations and MFI
Precision %CV in %populations of triplicates <i>Analysis: Mean %CV of 10 donors</i>	Passed: Triplicates had minimal variation in %populations
Cocktail Stability Aged cocktails vs fresh cocktail <i>Analysis: Relative %Difference of %populations and MFI</i>	Passed: Cocktails stored at 2-8°C in the dark for 4 weeks generated %populations and MFI values similar to the fresh cocktail

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