



Stable Recovery of Common and Rare CD Markers on Human Leukocytes in the Cyto-Chex® Blood Collection Tube Over Time.

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ABSTRACT

There is a limited time frame in which CD Marker expression can be accurately recovered from whole blood samples due to cell death. This forces samples to be processed for flow cytometry analysis soon after blood draw. The commercial blood collection tube Cyto-Chex® BCT stabilizes TBNK markers for monitoring HIV progression by flow cytometry for 14 days. This stabilizing tube has also been reported to extend the testing window for Zap-70 for up to five days. We examined the ability of Cyto-Chex BCT to similarly extend the analysis time frame for a wide range of common and rare CD markers used in leukemia/lymphoma classification and immunophenotyping. In this preliminary investigation, whole blood and cell lines spiked into whole blood drawn into Cyto-Chex BCT were used to mimic normal and abnormal patient samples, respectively. After collection, the samples were stored at room temperature and tested at 2, 72, and 168 hours post blood draw. The data show that Cyto-Chex BCT preserves the population positions of the lymphocytes, monocytes, and granulocytes. The data also show that unlike EDTA drawn samples, Cyto-Chex BCT drawn samples do not have non-specific antibody binding due to cell death. Finally, the data show that recovery of the majority of human CD markers tested, both extracellular and intracellular, can be achieved for at least 7 days. This allows for samples to be batched and processed at the discretion of the local testing lab or shipped to a central facility or contract research organization (CRO) for more advanced testing, without jeopardizing accurate CD marker recovery.

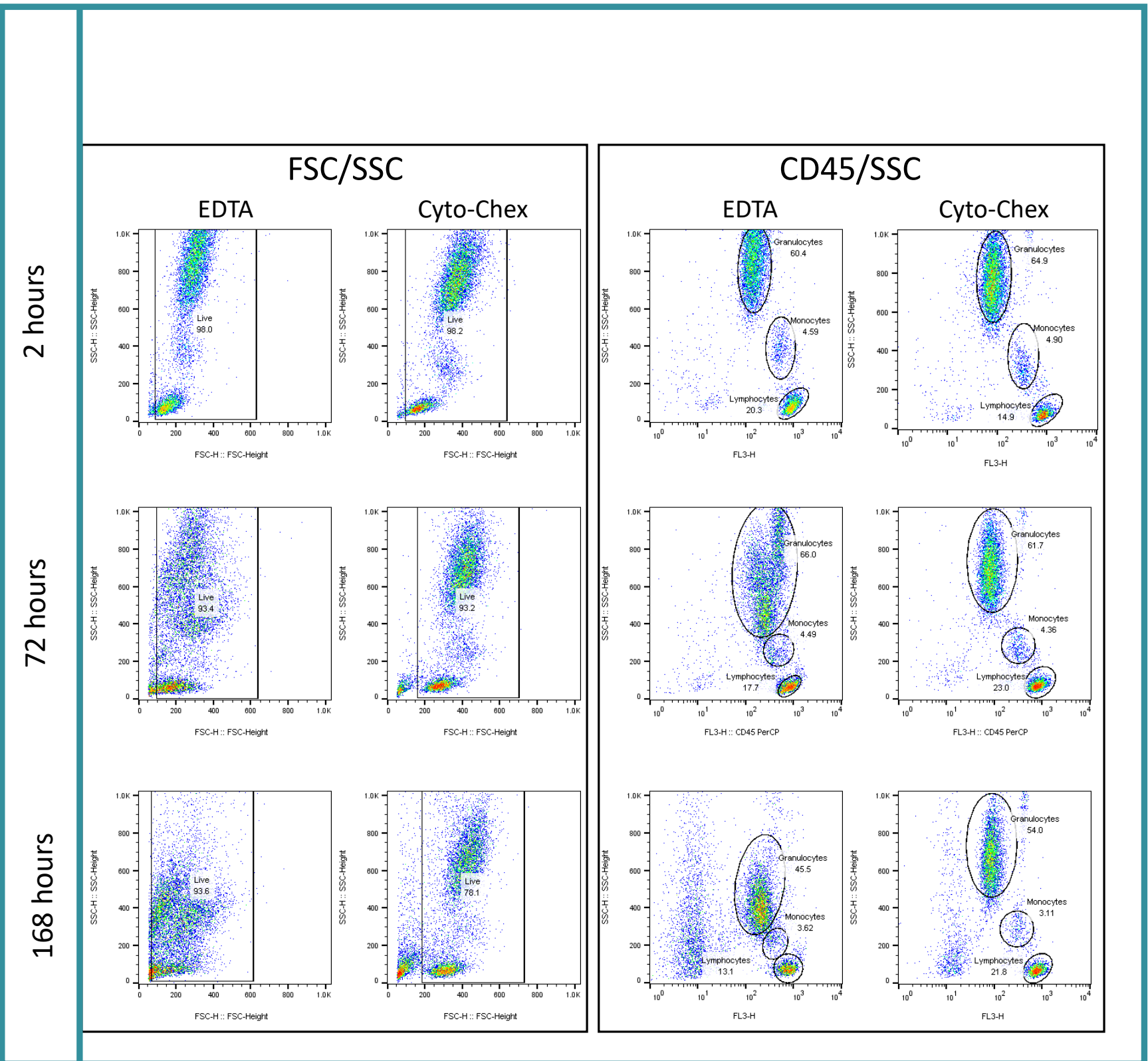
HYPOTHESIS

Cyto-Chex BCT has been shown to stabilize CD3, CD4, CD8, CD19, CD16/CD56 in human whole blood for 14 days. Cyto-Chex BCT can stabilize common and rare CD markers beyond the TBNK panel to 7 days post draw.

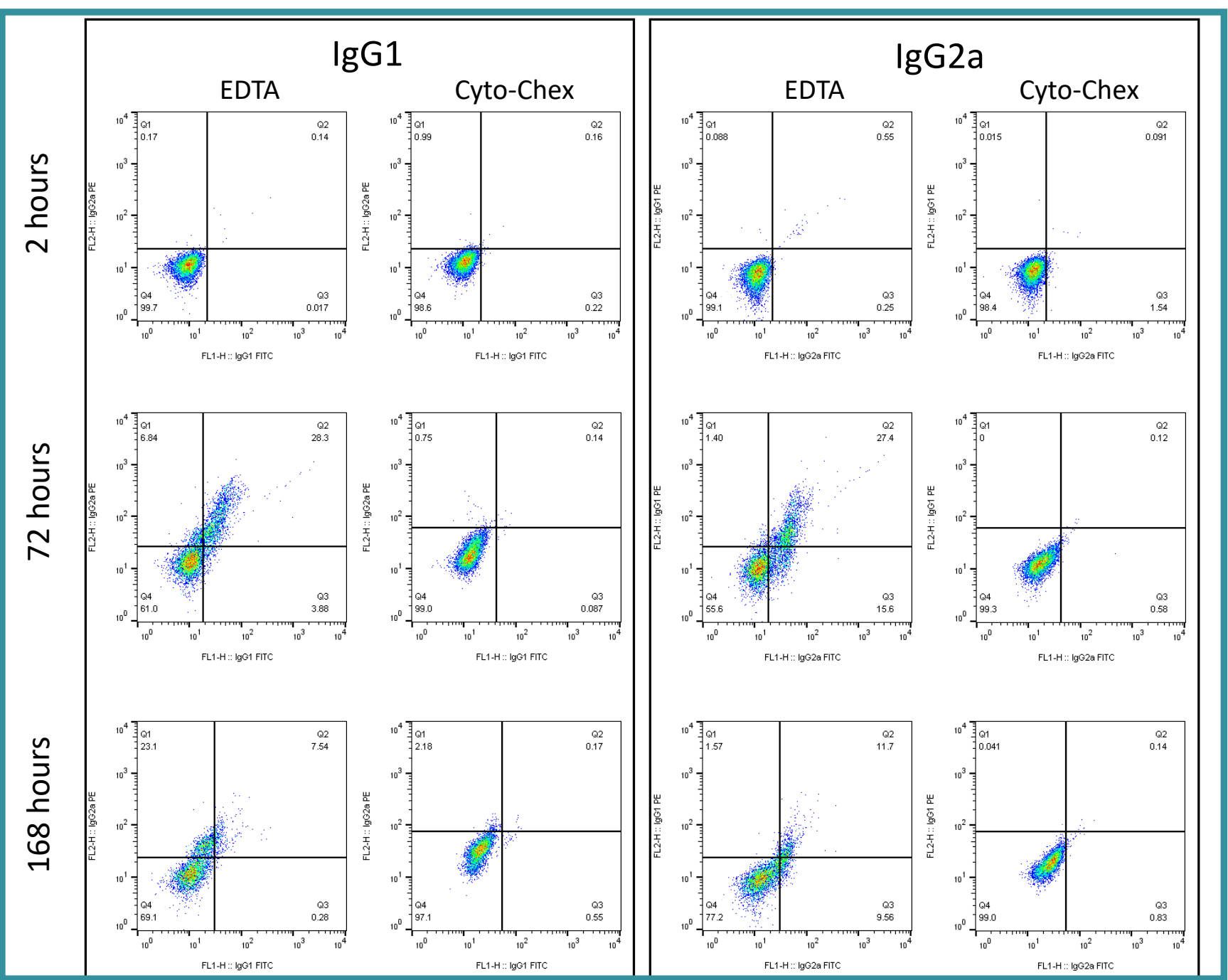
MATERIALS AND METHODS

Whole blood was collected from normal human donors and stored for the indicated time points at 22 °C. Cell lines possessing rare CD markers were added to the indicated samples to mimic abnormal patient blood. 100 µL of sample was stained with 5 µL to 10 µL of the indicated antibody for 20 minutes. The red blood cells were removed by addition of BD FACS™ Lysing Solution for 10 minutes and the samples were washed twice in PBS w/3% BSA. The stained samples were then run on a FACSCalibur™ and the data files analyzed using FlowJo® V10.

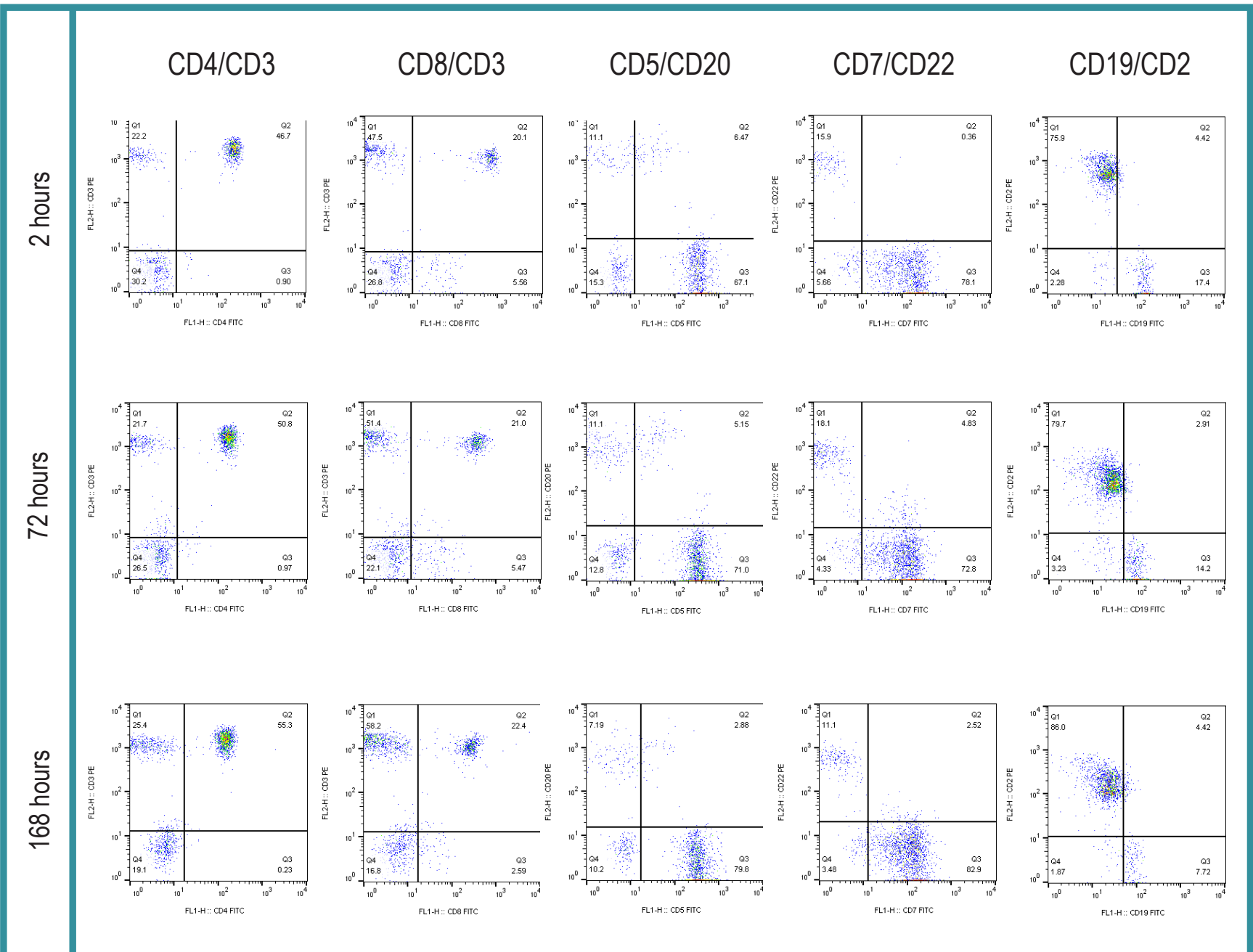
POPULATION SEPARATION OF CYTO-CHEX BCT COMPARED TO EDTA



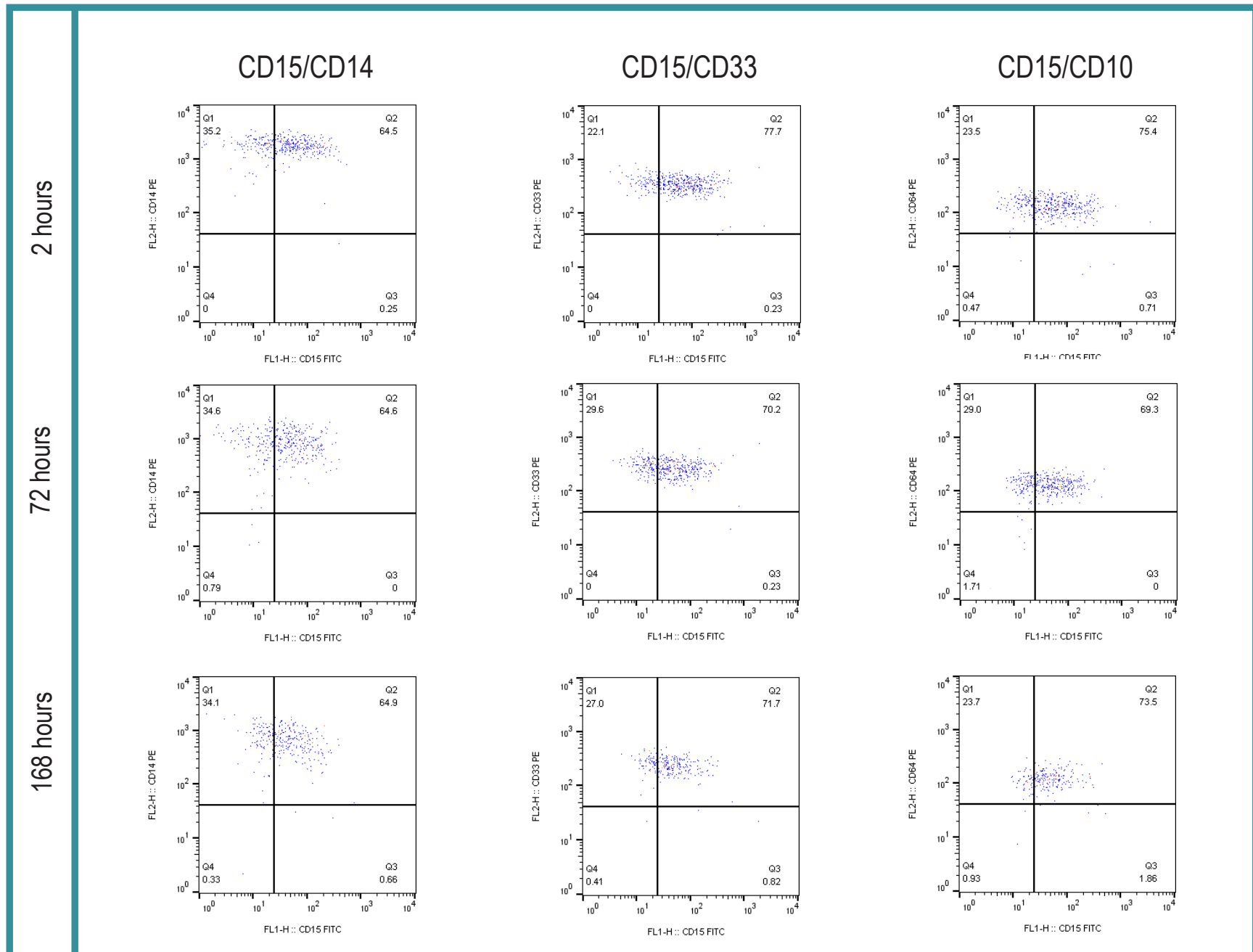
ISOTYPE CONTROLS



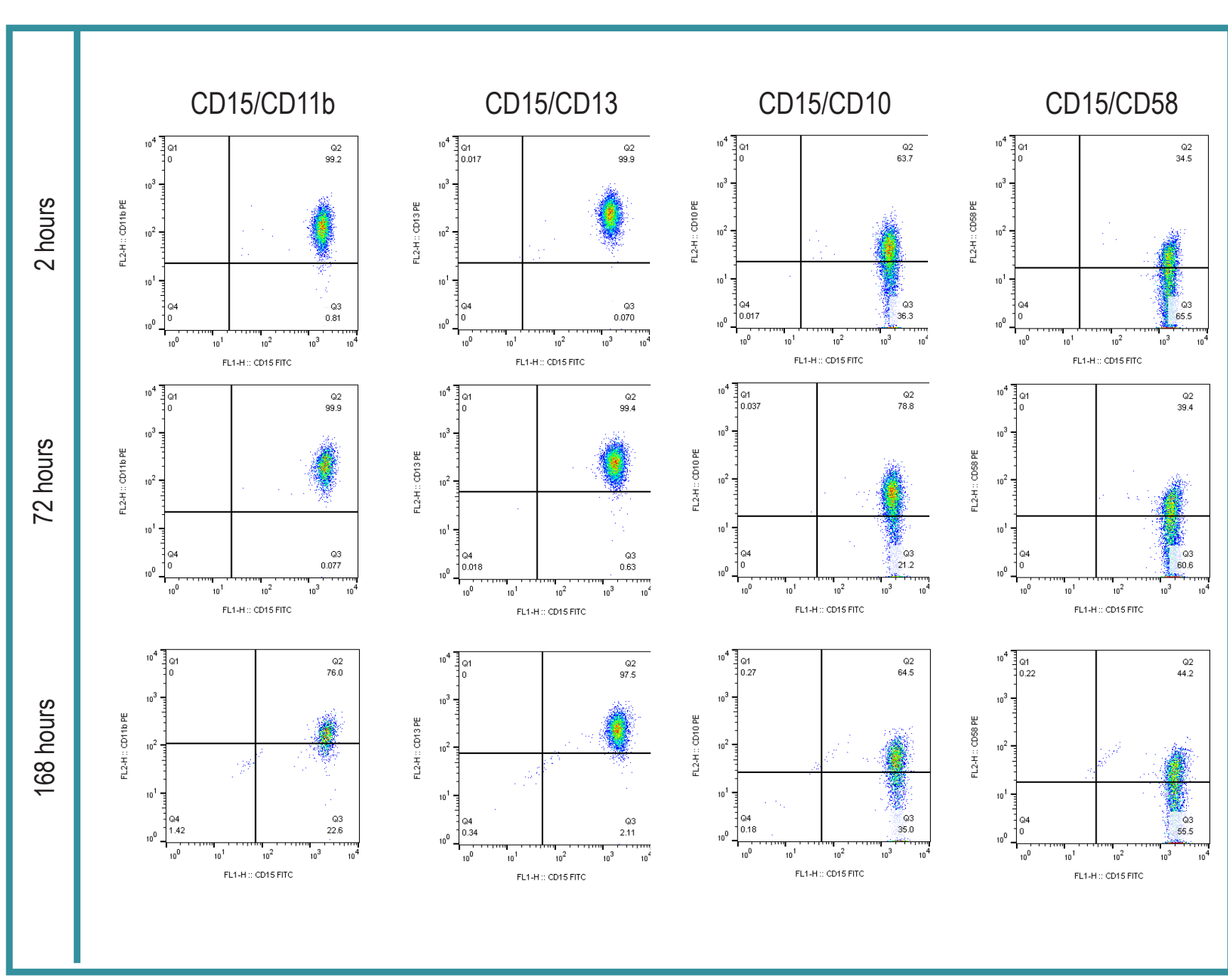
COMMON HUMAN LYMPHOCYTES STABILITY



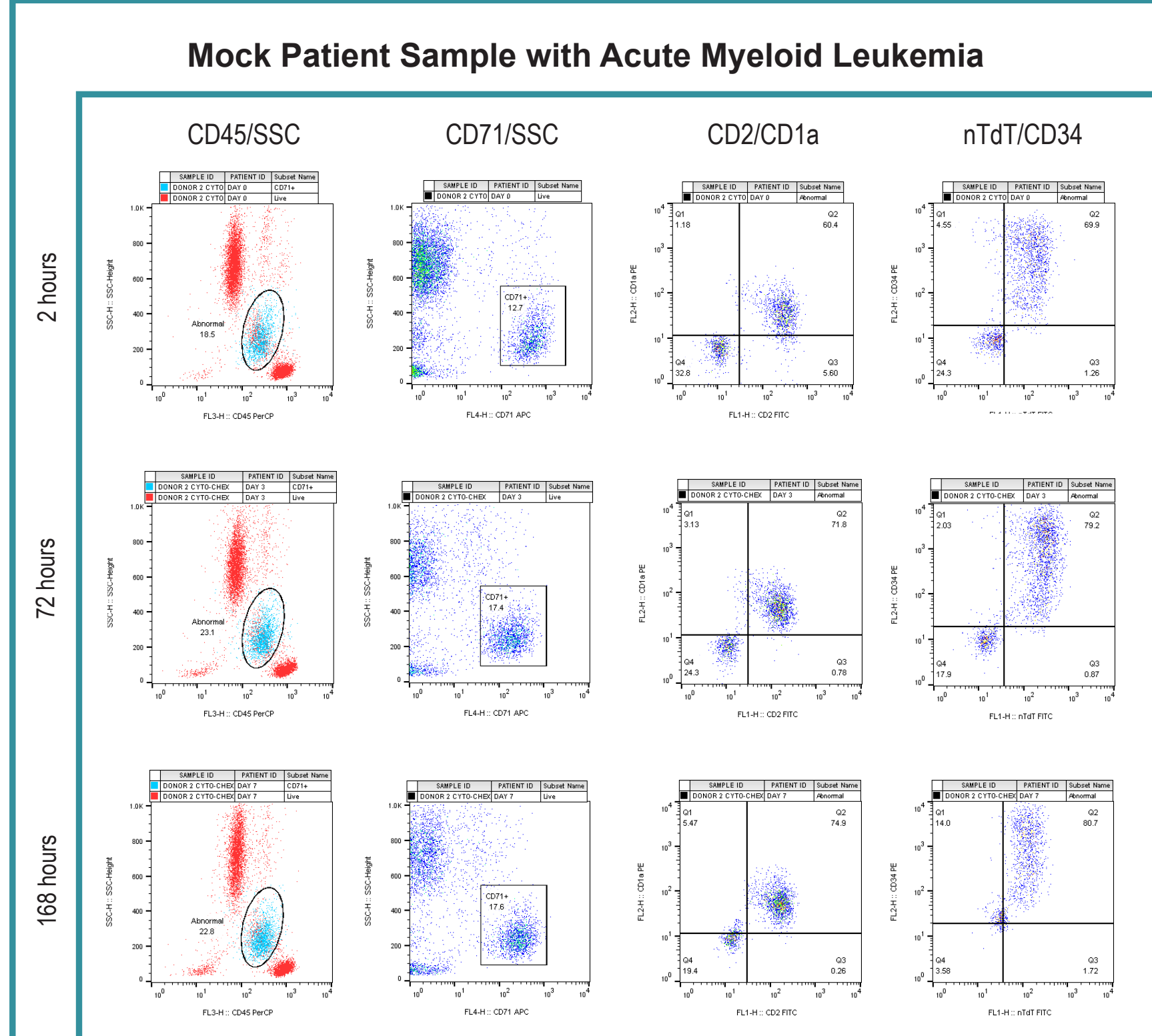
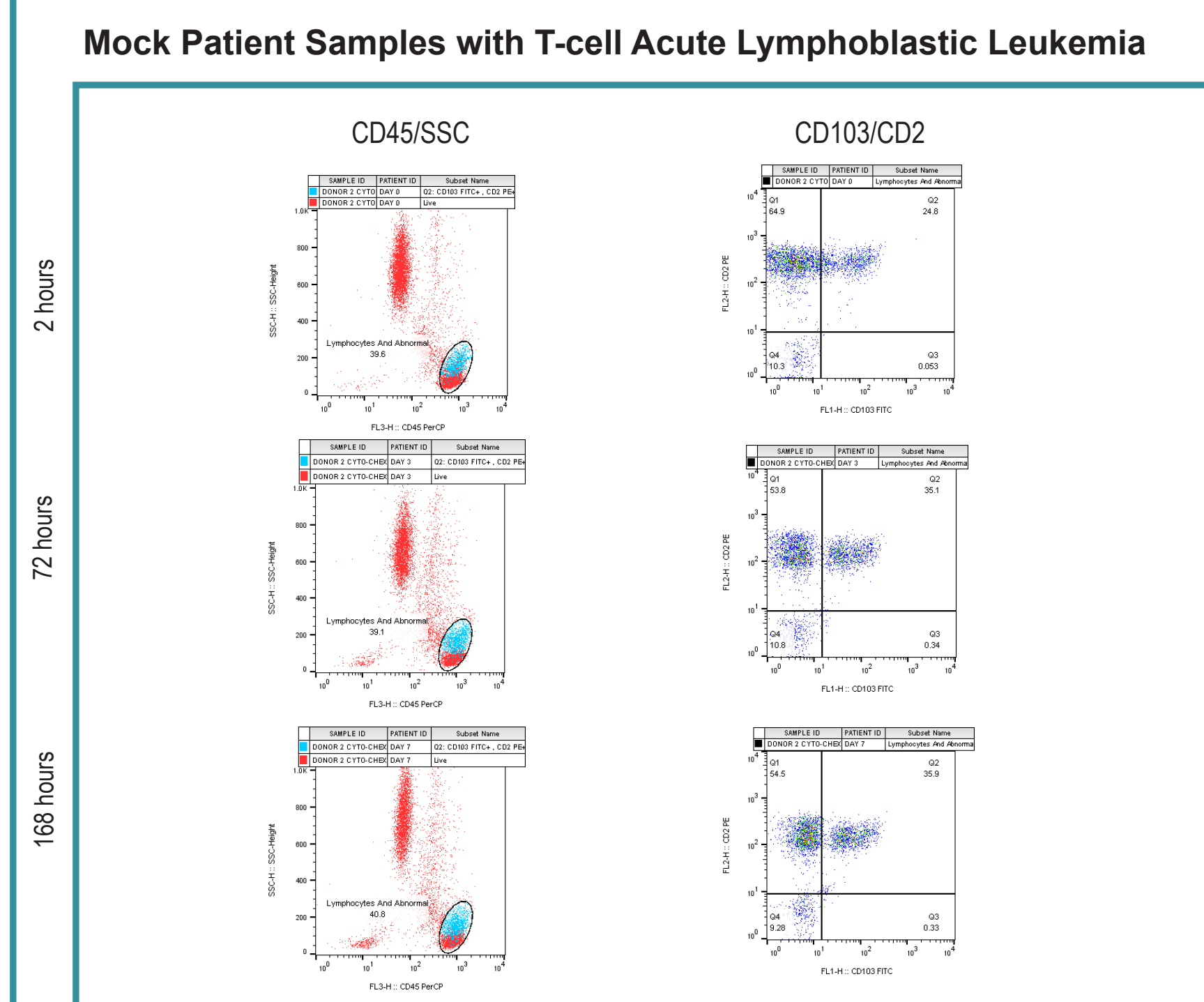
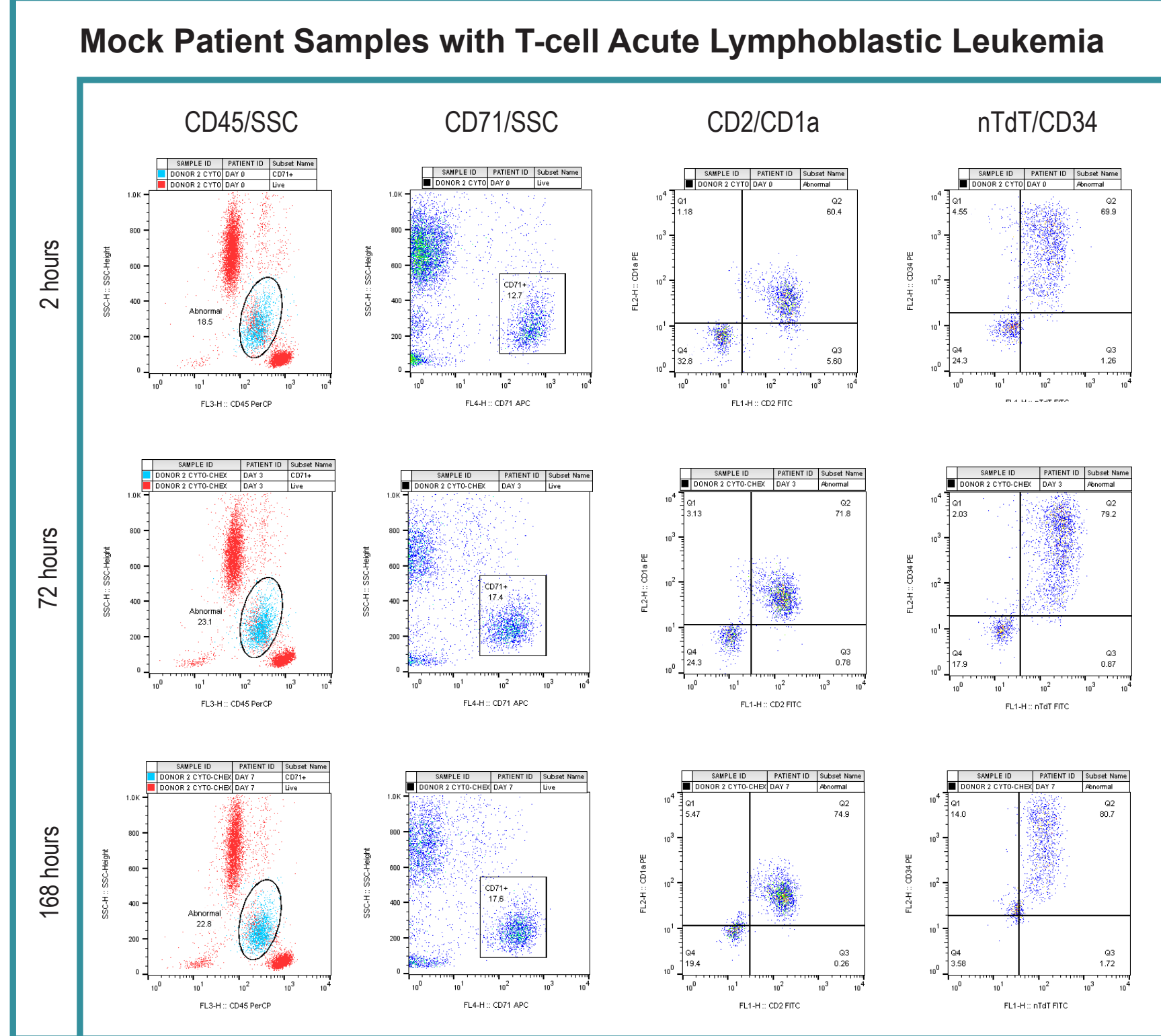
COMMON MONOCYTE RECOVERY



COMMON GRANULOCYTE RECOVERY



RARE CD MARKER RECOVERY



SUMMARY

CD markers tested for recovery in Cyto-Chex BCT 7 days post draw

	CD Marker	Type of marker	Antibody Clone		CD Marker	Type of marker	Antibody Clone
1	CD1a	Rare	SK9	19	CD25	Common	2A3
2	CD2	Common	S5.2	20	CD26	Common	L272
3	CD3	Common	SK7	21	CD33	Common	P67.6
4	CD4	Common	SK3	22	CD34	Rare	8G12
5	CD5	Common	L17F12	23	CD38	Common	HB7
6	CD7	Common	M-T701	24	CD45	Common	2D1
7	CD8	Common	SK1	25	CD56	Common	MY31
8	CD10	Common	HI10a	26	CD57	Common	HNK-1
9	CD11b	Common	ICRF44	27	CD58	Common	L306.4
10	CD11c	Common	S-HCL-3	28	CD64	Common	10.1
11	CD13	Common	L138	29	CD71	Rare	L01.1
12	CD14	Common	MpP9	30	CD103	Rare	Ber-ACT8
13	CD15	Common	MMA	31	CD117	Rare	104D2
14	CD16	Common	B73.1	32	CD123	Rare	9F5
15	CD19	Common	4G7	33	TdT	Rare	XHTdT
16	CD20	Common	L27	34	TCRab	Common	WT31
17	CD22	Common	S-HCL-1	35	TCRgd	Common	11F2
18	CD23	Common	M-L233	36	HLA-DR	Common	L243

CONCLUSION

Cyto-Chex BCT stabilizes white blood cells in whole blood and allows the recovery of a wide range of common and rare CD markers at seven days post draw.

- Cyto-Chex BCT maintains superior population separation compared to EDTA
- Cyto-Chex prevents non-specific binding of isotype controls
- Recovery of common CD markers on human lymphocytes, monocytes and granulocytes is maintained throughout 7 days post draw
- Recovery of rare CD markers is maintained throughout 7 days post draw