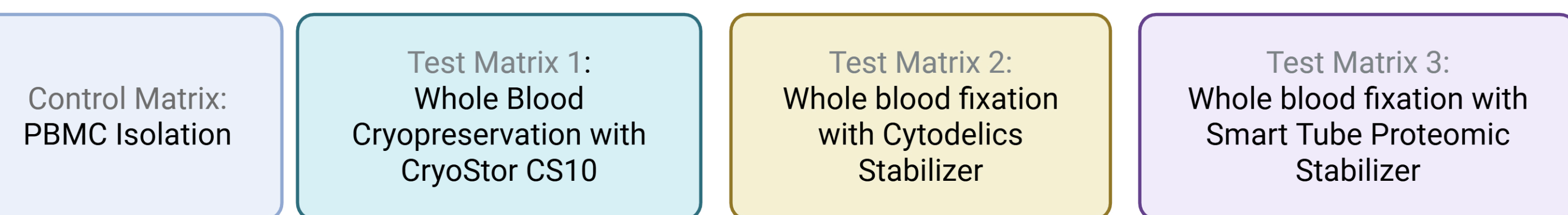


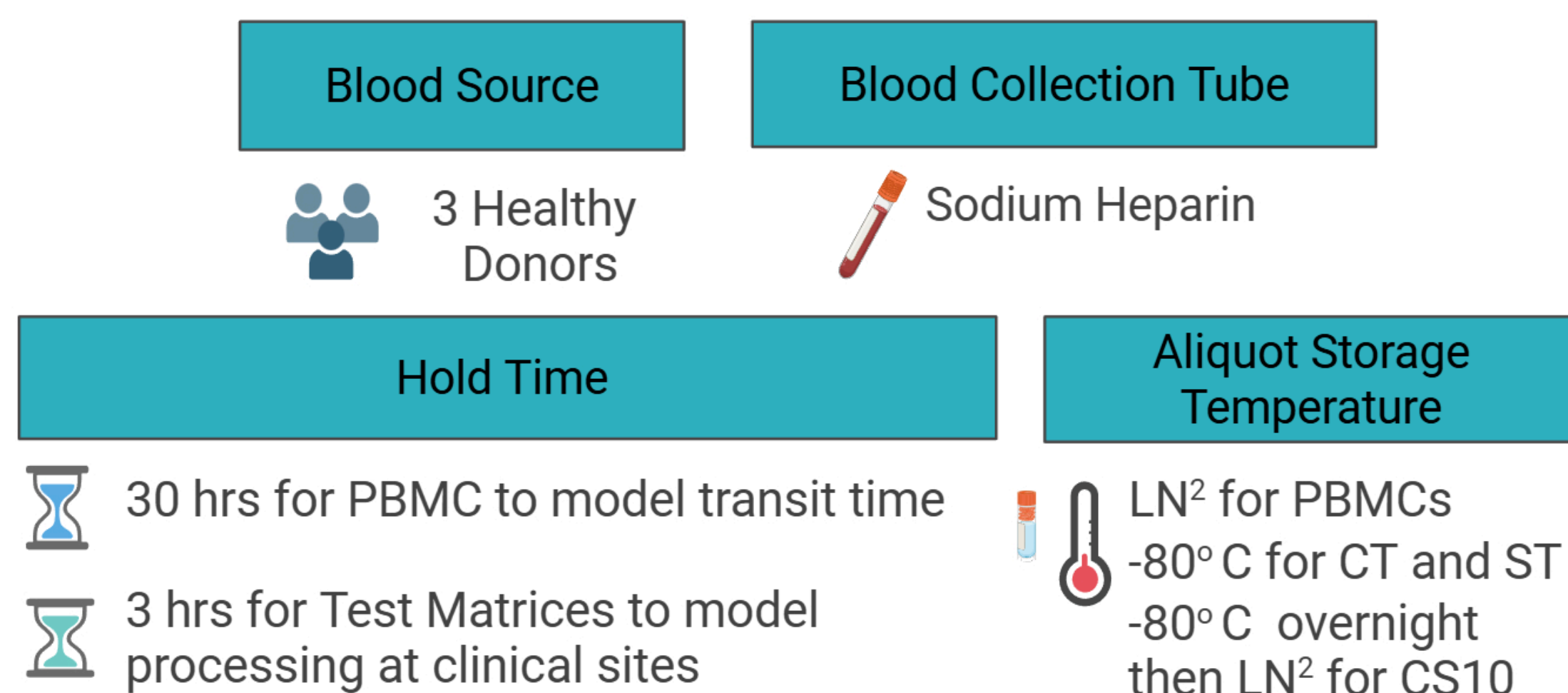
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Clinical Trial Challenge: Specimen Stability for Flow Cytometric Analysis

- Main Challenge** Blood Volume Restrictions and Specimen Integrity for Flow Cytometry Analysis
- Many clinical sites lack the expertise and/or infrastructure required for PBMC isolation, cryopreservation and shipment
 - Standard PBMC isolation requires a large volume of blood
- Feasibility Study** Evaluation of 3 whole blood preservation products as alternatives to PBMC isolation



Study Design



Sample Analysis

- Samples were evaluated after 1-week of storage by a 26-color spectral flow cytometric method for T cell subsets.
- The variance (%CV) was calculated between the results generated with each Test Matrix and the results from the PBMC sample processed at 30 hours post-collection.
- The resolution of each subset was scored as comparable, improved, or lower.

Table 1: The results from each of the Test Matrices compared to PBMC.

Red: %CV>25, Dark Blue: Improved Resolution, Light Blue: Comparable resolution, Orange: Lower resolution.

Population	Donor	PBMC				Cryostor CS10				Cytodelics				Smart Tube			
		% Pop	% Pop	% CV	Staining Pattern	% Pop	% CV	Staining Pattern	% Pop	% CV	Staining Pattern	% Pop	% CV	Staining Pattern			
CD4 ⁺ T cells	724	76.38	76.03	0.32	Comparable Resolution	69.56	6.61	Comparable Resolution	70.93	5.23	Comparable Resolution	61.94	18.85	Comparable Resolution			
	514	47.37	63.02	20.05	Improved Resolution	44.84	3.88	Comparable Resolution	61.94	18.85	Comparable Resolution	61.94	18.85	Comparable Resolution			
	780	65.14	46.49	23.63	Improved Resolution	62.94	2.43	Lower Resolution	46.22	24.03	Lower Resolution	46.22	24.03	Lower Resolution			
CD4 ⁺ Naive	724	52.37	42.95	13.98	Improved Resolution	37.26	23.84	Lower Resolution	37.75	22.94	Lower Resolution	40.25	31.92	Lower Resolution			
	514	63.72	38.92	34.17	Improved Resolution	47.63	20.44	Lower Resolution	40.25	31.92	Lower Resolution	40.25	31.92	Lower Resolution			
	780	41.18	55.57	21.03	Improved Resolution	36.70	8.14	Lower Resolution	50.17	13.92	Lower Resolution	50.17	13.92	Lower Resolution			
CD4 ⁺ Central Memory	724	24.96	29.64	12.12	Improved Resolution	45.98	41.90	Lower Resolution	51.40	48.97	Lower Resolution	48.12	42.18	Lower Resolution			
	514	26.01	33.01	16.77	Improved Resolution	40.89	31.46	Lower Resolution	48.12	42.18	Lower Resolution	48.12	42.18	Lower Resolution			
	780	34.95	37.06	4.14	Improved Resolution	35.24	0.58	Lower Resolution	47.90	22.11	Lower Resolution	47.90	22.11	Lower Resolution			
CD4 ⁺ Effector Memory	724	18.41	23.15	16.13	Improved Resolution	15.18	13.60	Lower Resolution	9.85	42.84	Lower Resolution	9.85	42.84	Lower Resolution			
	514	8.66	24.46	67.47	Improved Resolution	10.55	13.91	Lower Resolution	9.52	6.69	Lower Resolution	9.52	6.69	Lower Resolution			
	780	20.53	6.66	72.14	Improved Resolution	25.21	14.47	Lower Resolution	1.74	119.32	Lower Resolution	1.74	119.32	Lower Resolution			
CD4 ⁺ TEMRA	724	4.26	4.21	0.83	Improved Resolution	1.64	62.80	Lower Resolution	1.00	87.65	Lower Resolution	2.14	17.86	Lower Resolution			
	514	1.66	3.69	53.66	Improved Resolution	0.89	42.70	Lower Resolution	2.14	17.86	Lower Resolution	2.14	17.86	Lower Resolution			
	780	3.45	0.79	88.72	Improved Resolution	2.70	17.25	Lower Resolution	0.18	127.40	Lower Resolution	0.18	127.40	Lower Resolution			
CD28 ⁺ PD-1 ⁺ CD4 ⁺	514	13.35	12.57	4.26	Comparable Resolution	16.29	14.03	Lower Resolution	16.73	15.89	Lower Resolution	16.73	15.89	Lower Resolution			
	724	10.10	9.97	0.92	Comparable Resolution	10.01	0.63	Lower Resolution	12.27	13.72	Lower Resolution	12.27	13.72	Lower Resolution			
	780	17.17	15.03	9.40	Comparable Resolution	20.12	11.19	Lower Resolution	9.71	39.25	Lower Resolution	9.71	39.25	Lower Resolution			
CD28 ⁺ TIGIT ⁺ CD4 ⁺	724	10.05	9.48	4.13	Comparable Resolution	16.06	32.55	Lower Resolution	8.82	9.22	Lower Resolution	8.82	9.22	Lower Resolution			
	514	43.14	38.34	8.33	Comparable Resolution	57.98	20.75	Lower Resolution	54.46	16.40	Lower Resolution	54.46	16.40	Lower Resolution			
	780	4.14	12.86	72.54	Comparable Resolution	10.20	59.76	Lower Resolution	13.10	73.50	Lower Resolution	13.10	73.50	Lower Resolution			
CD28 ⁺ KLRG1 ⁺ CD4 ⁺	724	7.55	4.31	38.63	Comparable Resolution	13.00	37.51	Lower Resolution	6.44	11.22	Lower Resolution	6.44	11.22	Lower Resolution			
	514	6.19	6.26	0.80	Comparable Resolution	6.26	0.80	Lower Resolution	5.68	6.08	Lower Resolution	5.68	6.08	Lower Resolution			
	780	11.25	7.04	32.55	Comparable Resolution	14.18	16.29	Lower Resolution	4.17	64.93	Lower Resolution	4.17	64.93	Lower Resolution			
Tregs	724	5.02	13.36	64.17	Comparable Resolution	5.62	7.97	Lower Resolution	13.16	63.32	Lower Resolution	13.16	63.32	Lower Resolution			
	514	19.34	19.34	0.00	Comparable Resolution	25.03	18.14	Lower Resolution	23.64	14.15	Lower Resolution	23.64	14.15	Lower Resolution			
	780	47.43	32.47	26.48	Comparable Resolution	50.66	4.66	Lower Resolution	31.63	28.26	Lower Resolution	31.63	28.26	Lower Resolution			
CD8 ⁺ T cells	724	30.13	48.42	32.93	Comparable Resolution	32.37	5.07	Lower Resolution	49.30	34.13	Lower Resolution	49.30	34.13	Lower Resolution			
	514	9.52	10.06	3.90	Comparable Resolution	3.01	73.48	Lower Resolution	8.51	7.92	Lower Resolution	8.51	7.92	Lower Resolution			
	780	42.17	51.35	13.88	Improved Resolution	25.27	35.44	Lower Resolution	43.32	1.90	Lower Resolution	43.32	1.90	Lower Resolution			
CD8 ⁺ Naive	724	53.95	45.01	12.78	Improved Resolution	64.55	12.65	Lower Resolution	31.88	36.36	Lower Resolution	31.88	36.36	Lower Resolution			
	514	3.00	4.47	27.83	Improved Resolution	0.15	127.95	Lower Resolution	0.24	120.47	Lower Resolution	0.24	120.47	Lower Resolution			
	780	17.31	3.40	94.99	Improved Resolution	50.79	69.53	Lower Resolution	14.60	12.01	Lower Resolution	14.60	12.01	Lower Resolution			
CD8 ⁺ Central Memory	724	3.11	19.26	102.10	Improved Resolution	12.53	85.18	Lower Resolution	58.19	127.07	Lower Resolution	58.19	127.07	Lower Resolution			
	514	19.93	20.48	1.92	Comparable Resolution	15.23	18.90	Lower Resolution	4.69	87.54	Lower Resolution	4.69	87.54	Lower Resolution			
	780	11.04	5.87	43.24	Improved Resolution	19.12	37.89	Lower Resolution	26.72	58.73	Lower Resolution	26.72	58.73	Lower Resolution			
CD8 ⁺ Effector Memory	724	6.05	10.28	36.63	Improved Resolution	7.65	16.52	Lower Resolution	5.87	2.14	Lower Resolution	5.87	2.14	Lower Resolution			
	514	67.53	65.01	2.69	Comparable Resolution	81.64	13.38	Lower Resolution	86.61	17.51	Lower Resolution	86.61	17.51	Lower Resolution			
	780	29.46	39.37	20.36	Comparable Resolution	4.81	101.72	Lower Resolution	15.36	44.49	Lower Resolution	15.36	44.49	Lower Resolution			
CD8 ⁺ TEMRA	514	36.89	25.42	26.03	Comparable Resolution	15.26	58.66	Lower Resolution	3.92	114.25	Lower Resolution	3.92	114.25	Lower Resolution			
	724	12.24	3.29	81.50	Comparable Resolution	15.78	17.87	Lower Resolution	5.39	54.95	Lower Resolution	5.39	54.95	Lower Resolution			
	780	1.99	10.81	97.45	Improved Resolution	7.55	82.42	Lower Resolution	19.11	114.75	Lower Resolution	19.11	114.75	Lower Resolution			
CD28 ⁺ PD-1 ⁺ CD8 ⁺	724	17.58	17.68	0.40	Improved Resolution	20.86	12.07	Comparable Resolution	17.83	1.00	Comparable Resolution	17.83	1.00	Comparable Resolution			
	514	19.04	22.55	11.94	Comparable Resolution	20.26	4.39	Lower Resolution	21.54	8.71	Lower Resolution	21.54	8.71	Lower Resolution			
	780	17.13	5.11	76.43	Improved Resolution	19.75	10.05	Lower Resolution	6.35	64.93	Lower Resolution	6.35	64.93	Lower Resolution			
CD28 ⁺ TIGIT ⁺ CD8 ⁺	724	6.89	19.34	67.13	Improved Resolution	6.16	7.91	Comparable Resolution	21.39	72.51	Lower Resolution	21.39	72.51	Lower Resolution			
	514	49.25	46.68	3.79	Comparable Resolution	56.67	9.91	Lower Resolution	59.41	13.22	Lower Resolution	59.41	13.22	Lower Resolution			
	780	18.76	6.58	67.98	Improved Resolution	28.46	29.05	Lower Resolution	10.46	40.17	Lower Resolution	10.46	40.17	Lower Resolution			
CD28 ⁺ KLRG1 ⁺ CD8 ⁺	724	4.96	17.26	78.28	Improved Resolution	11.19	54.55	Lower Resolution	29.05	100.17	Lower Resolution	29.05	100.17	Lower Resolution			

Results

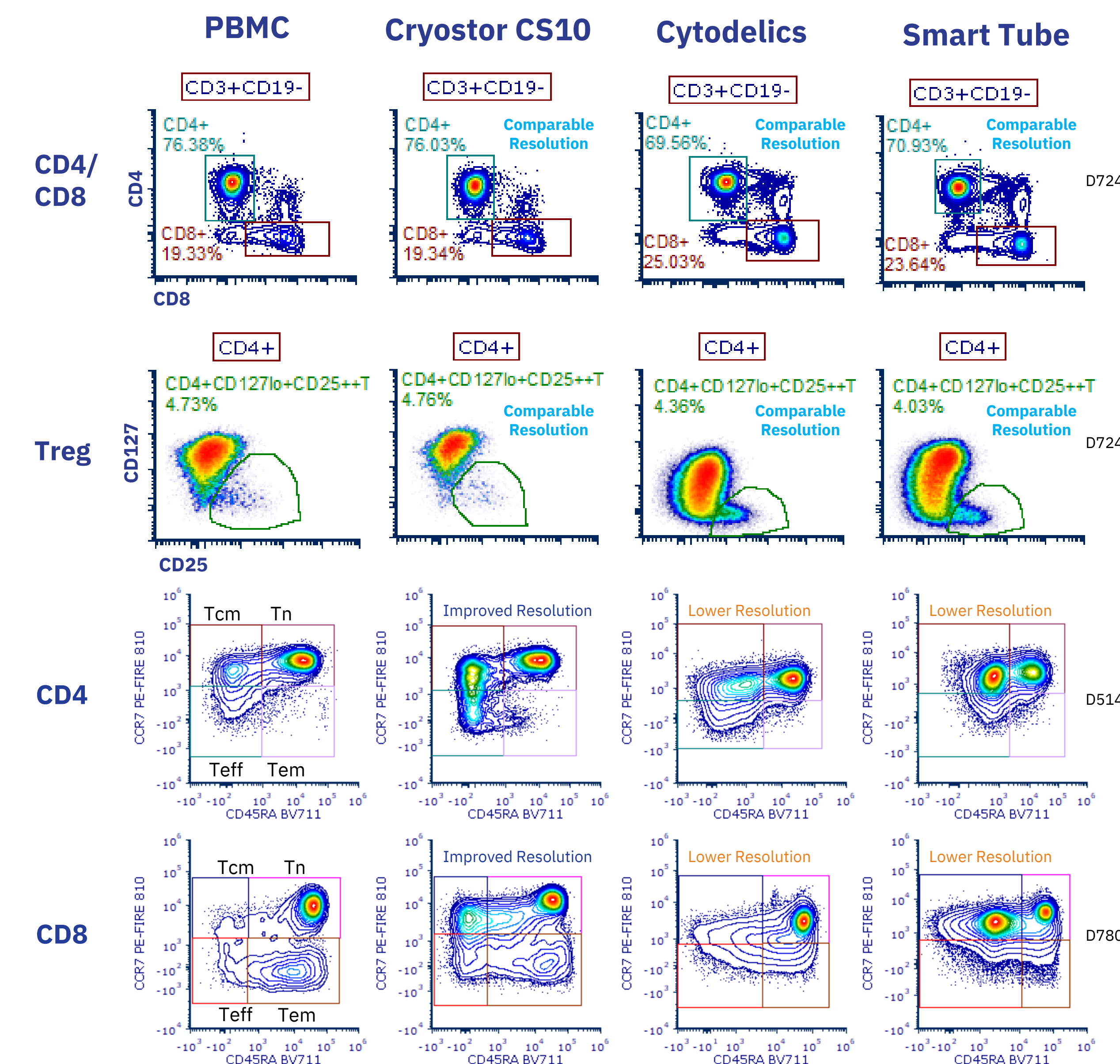


Figure 1: Representative flow cytometry plots showing total CD4, total CD8, T Regulatory (Treg), and CD4 and CD8 naïve and memory cells, as labeled. Naïve (Tn), Central Memory T cells (Tcm), Effector Memory T cells (Teff), Terminally Differentiated Effector Memory T cells (Tem). Blood donor ID is listed on the right side.

Conclusions

- Study Outcomes** Pilot Study Results of Test Matrices
- Based on this pilot study, whole blood preservation using Cryostor CS10 is the preferred matrix when traditional PBMC cryopreservation is not possible due to blood volume restrictions or logistical challenges.
 - Cell population comparisons should be confined to samples preserved in the same matrix.
 - Donor variability highlights the importance of including multiple donors in feasibility studies.
 - Cytodelics and Smart Tube matrices contain fixatives; therefore, antibody re-titration may improve resolution.