Preserving Rat Whole Blood for Delayed Cell Surface Antigen Analysis by Flow Cytometry: a Potential Stress-Reliever in Pre-Clinical Research

Michael Paton, Atreyee Sims, Dominic Warrino, Masood Khan – KCAS Bioanalytical Services, Kansas City, KS 66216

INTRODUCTION

Flow cytometric analysis of cell surface markers is a functionally-informative technique, especially in monitoring the drug induced toxicity and safety assessment in test animals [1]. Cryopreservation of PBMC and/or overnight shipping of samples to the Flow facility is often used, which may have adverse effects on immune monitoring assays using flow cytometry and related techniques [2].

Typically, confidence in the accuracy of results from the live-cell based techniques decreases rapidly with time after sample collection. Obtaining the reliable results from fresh blood samples necessitates the close vicinity of the analytical lab to the in-life research facility, and speedy sample transport between the two. A method that successfully preserved CD markers would solve this logistic obstacle, and potentially reduce costs by allowing samples to be collected and then batch analyzed. In this study we evaluated the ability of Streck Cell Preservative™ to retain intact CD markers on rat whole blood samples over time.

METHOD

- Rat whole blood samples were collected in K_2EDTA Vacutainer.
- Freshly collected samples (n=3) were preserved by diluting 1:1 with Streck Cell Preservative™ within 3 hours of collection.
- Samples were stained using a Lyse/No-Wash protocol and acquired on a BD FACSCalibur.
- Preserved samples were analyzed at Baseline, Day 1, and Day 5.
- Unpreserved samples were also tested at Baseline and Day 5 for scatter comparison.

GATING STRATEGY

- The lymphocyte population was identified in a CD45-PE-Cy7 (FL3) by Side Scatter dual-parameter histogram. Two plots were used to subdivide the lymphocyte population:

•CD45RA-FITC vs. CD3-APC (FL1 vs. FL4)

– T cell percentage determined as Lymphocyte+ CD3+ CD45RA-

– B cell percentage determined as Lymphocyte+ CD45RA+ CD3-

•CD161a-PE vs. CD3-APC (FL2 vs. FL4).

– NK cell percentage determined as Lymphocyte+ CD161a+ CD3-

Table 1: Flurochrome-Conjugated Antibodies

Antibody	Clone	Isotype
Anti-Rat CD3 - APC	1F4	mIgM
Anti-Rat CD45RA - FITC	OX-33	mIgG1
Anti-Rat CD161a - PE	10/78	mIgG1
Anti-Rat CD45 - PE-Cy™7	OX-1	mIgG1

RESULTS

Table 2: *Preserved* Sample %CV Across Time Points

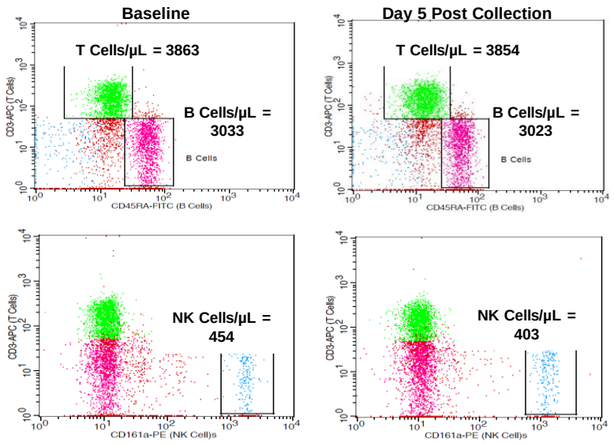
		Lymphocytes	T Cells		B Cells		NK Cells	
Sample ID	Time Point	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)
BW0001	Pres-Day0	10056.78	38.4	3863.08	30.2	3033.08	4.5	454.36
	Pres-Day 1	9455.38	39.9	3773.64	29.0	2741.64	4.5	425.28
	Pres-Day 5	9792.18	39.4	3854.26	30.9	3023.8	4.1	403.98
	%CV	**3.09**	**1.93**	**1.29**	**3.16**	**5.65**	**5.01**	**5.91**
BW0004	Pres-Day0	12491.76	28.5	3554.46	25.7	3212.40	7.5	932.44
	Pres-Day 1	11491.04	29.8	3422.18	22.0	2526.28	7.0	808.28
	Pres-Day 5	11391.86	29.3	3333.18	24.5	2789.96	7.8	893.42
	%CV	**5.16**	**2.30**	**3.24**	**7.92**	**12.17**	**5.44**	**7.23**
BW3005	Pres-Day0	10735.92	39.4	4224.52	35.1	3764.18	1.7	187.30
	Pres-Day 1	11126.44	43.1	4790.88	32.3	3588.50	1.9	215.02
	Pres-Day 5	10436.42	40.5	4230.46	34.0	3543.50	2.0	204.26
	%CV	**3.21**	**4.62**	**7.37**	**4.19**	**3.21**	**6.36**	**6.91**

Table 3: % Change in *Preserved* Populations Over Time

		Lymphocytes	T Cells		B Cells		NK Cells	
Sample ID	Time Point	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)
BW0001	Pres-Day0	10056.78	38.4	3863.08	30.2	3033.08	4.5	454.36
	Pres-Day 1	9455.38	39.9	3773.64	29.0	2741.64	4.5	425.28
	Pres-Day 5	9792.18	39.4	3854.26	30.9	3023.8	4.1	403.98
% Change, Day0 - Day5		**-2.63**	**2.47**	**-0.23**	**2.39**	**-0.31**	**-8.63**	**-11.09**
% Change, Day1 - Day5		**3.56**	**-1.38**	**2.14**	**6.48**	**10.29**	**-8.22**	**-5.01**
BW0004	Pres-Day0	12491.76	28.5	3554.46	25.7	3212.40	7.5	932.44
	Pres-Day 1	11491.04	29.8	3422.18	22.0	2526.28	7.0	808.28
	Pres-Day 5	11391.86	29.3	3333.18	24.5	2789.96	7.8	893.42
% Change, Day0 - Day5		**-8.81**	**2.85**	**-6.23**	**-4.78**	**-13.15**	**5.09**	**-4.18**
% Change, Day1 - Day5		**-0.86**	**-1.75**	**-2.60**	**11.42**	**10.44**	**11.52**	**10.53**
BW3005	Pres-Day0	10735.92	39.4	4224.52	35.1	3764.18	1.7	187.30
	Pres-Day 1	11126.44	43.1	4790.88	32.3	3588.50	1.9	215.02
	Pres-Day 5	10436.42	40.5	4230.46	34.0	3543.50	2.0	204.26
% Change, Day0 - Day5		**-2.79**	**3.02**	**0.14**	**-3.17**	**-5.86**	**12.64**	**9.05**
% Change, Day1 - Day5		**-6.20**	**-5.85**	**-11.70**	**5.27**	**-1.25**	**1.55**	**-5.00**

Percent Change calculated as = (New Value - Old Value) / (Old Value) * 100

Fig 1: BW0001 *Preserved* Rat Whole Blood T B NK Cells



RESULTS (CONT.)

Fig 2: BW3005 *Preserved* Rat Whole Blood Lymphocytes

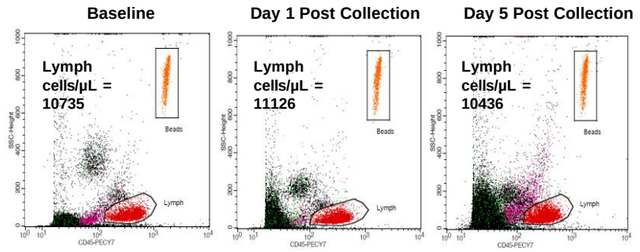


Fig 3: BW0001 *Preserved* 3-Part Differential Over Time

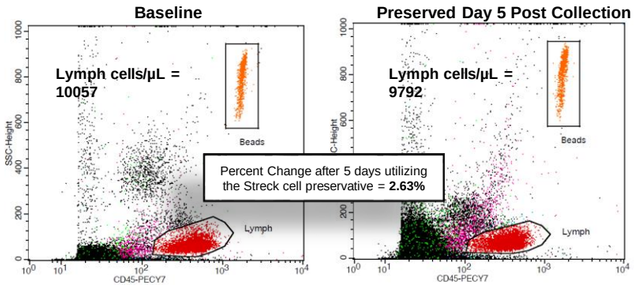


Fig 4: BW0001 ***Un-Preserved*** 3-Part Differential

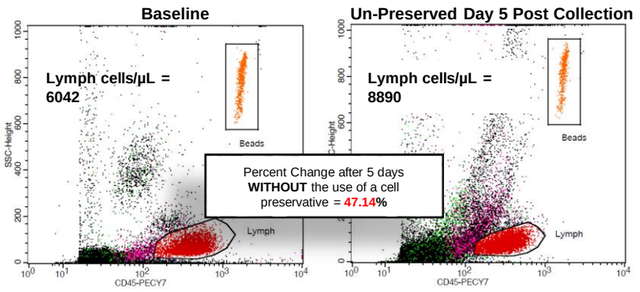
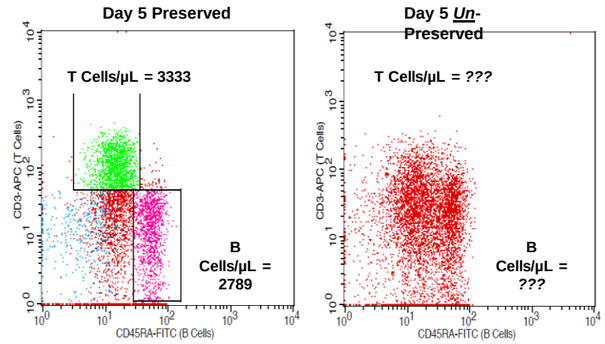


Table 4: Percent Change in ***Un-Preserved*** Populations

		Lymphocytes	T Cells		B Cells		NK Cells	
Sample ID	Time Point	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)	% Gated of Lymph	Absolute Count (cells/uL)
BW0001	Unpres-Day0	6041.98	39.0	2357.27	30.6	1849.66	4.5	271.45
	Unpres-Day 5	8890.30	28.3	2517.38	27.8	2472.44	4.9	433.57
% Change, Day0 - Day5		**47.14**	**-27.40**	**6.79**	**-9.15**	**33.67**	**8.69**	**59.72**
BW0004	Unpres-Day0	11307.08	32.9	3718.52	24.2	2736.42	8.0	900.17
	Unpres-Day 5	11087.84	18.6	2067.15	25.5	2828.04	8.6	947.81
% Change, Day0 - Day5		**-1.94**	**-43.33**	**-44.41**	**5.41**	**3.35**	**7.41**	**5.29**
BW3005	Unpres-Day0	10542.70	39.8	4194.95	33.1	3483.96	2.0	209.85
	Unpres-Day 5	10491.85	28.7	3008.38	30.9	3246.50	2.1	222.73
% Change, Day0 - Day5		**-0.48**	**-27.95**	**-28.29**	**-6.38**	**-6.82**	**6.53**	**6.14**

RESULTS (CONT.)

Fig 5: BW0004 Preserved Vs. Un-Preserved at Day 5



SUMMARY

- The %CV for each population over the three time points ranged from 1.3% to 12.2% amongst preserved samples.
- The Percent Change between Baseline and Day 5 ranged from 0.2% to 13.2% across all populations, percent gated as well as absolute counts, amongst preserved samples.
- In the Day 5 preserved samples, the sub-populations are still distinct. In contrast, after 5 days the un-preserved sample would be impossible to accurately gate, as the scatter has degraded into one indistinguishable mass. As a result, it was impossible to determine accurate results for the **un**-preserved samples after 5 days.

CONCLUSION

- This study demonstrated equivalent results generated 5 days after sample collection, which is more than ample time to transport samples to the preferred, and not just the closest, analytical laboratory.
- The ability to maintain the integrity of cell surface markers would also allow for samples to be batched and analyzed after a weekend, or holiday, and make re-analysis possible in the case of technical error, instrument failure, or shipping delay.
- All of these factors support the usefulness of the Streck Cell Preservative™ in pre-clinical immunophenotyping of rat whole blood samples, and its use may result in a better data set, and the potential for significant cost savings.

REFERENCES

1. McFarland D & Harkins KR, Flow cytometry in preclinical toxicology/safety assessment. *In* Flow Cytometry in Drug Discovery and Development, Eds. Litwin V & Marder P (John Wiley & Sons, Inc), pp 123- 200 (2011).

2. Maecker HT *et al*. Impact of cryopreservation on tetramer, cytokine flow cytometry, and ELISPOT. BMC Immunology, doi:10.1186/1471-2172-6-17 (2005).