Hydrogels Mimicking T, B, and NK Cells for Quality Assurance in Cell Therapy Development

Sarah Kotanchiyev, Swetha Gunturu, Amay Dankar, Subhanip Biswas, Louisa D'Lima, Armando Martinez, Kanwal Palla
Slingshot Biosciences, Inc.

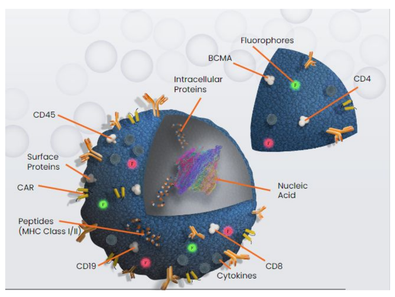
ABSTRACT

Hydrogel cell mimics aid in validating flow cytometry assays by minimizing the variability of traditionally used PBMC controls. Here, we present data using hydrogel cell mimics mimicking T, B, and NK cell subsets from healthy donor blood. We demonstrate consistent lot-to-lot reproducibility and stability against lyophilized and cryopreserved PBMC controls from different donors. The results show that use of hydrogel cell mimics provide a robust quality control for cell characterization and quantification to standardize and accelerate analytical development.

INTRODUCTION

Patient characterization is critical throughout the cell therapy life cycle, including post-market patient monitoring. Characterization or immunophenotyping assays measure T cells, B cells, and NK cells, but traditional healthy donor sourced cells are prone to variability between PBMC donors, which leads to a lack of reproducibility during assay development and verification.

Slingshot Biosciences aims to solve these problems by providing first-of-its-kind TBNK cell mimics. Our TruCytes™ Lymphocyte Subset control is a lyophilized mixture of hydrogel cell mimics engineered to mimic PBMCs or leukopak-based controls in terms of scatter, biomarker expression levels, and immune cell subset population frequencies.



The TruCytes™ Lymphocyte Subset hydrogel cell mimics use Slingshot Biosciences' proprietary technology to independently tune optical and biochemical parameters producing highly customizable controls.

Gating Scheme

Granulocytes (CD45) 3-7%	Monocytes (CD45) 9-19%	Lymphocytes (CD45) 78-86%		
	Classical Monocytes (CD45, CD14) 90-100%	T-Cells (CD45, CD3) 69-79%	B-Cells (CD45, CD19) 10-20%	Natural Killer (NK) (CD45, CD3-, CD19-) 6-16%
		T-Helper (CD45, CD3, CD4) 63-73%	T-Cytotoxic (CD45, CD3, CD8) 27-37%	Natural Killer (NK) (CD45, CD16, CD56) 90-100%

The populations included in TruCytes™ Lymphocyte Subset are T cells (CD3 and CD4/CD8), NK cells (CD16 and CD56), B cells (CD19), and Monocytes (CD14).

METHODOLOGY

- Lot-to-to Consistency was determined using two different lots of lyophilized and cryopreserved controls tested against two lots of TruCytes™ Lymphocyte Subset Controls on a Cytek® Aurora CS
- Fixation buffers from three different vendors were tested on TruCytes™ to evaluate their suitability for fixing cell mimics for immunofluorescent staining. The assessment focused on whether the fluorescent antibodies could specifically detect their cognate antigens in the fixed cells.
- Two different RBC lysis protocols were tested on TruCytes™ to assess the compatibility and effectiveness of the 1X RBC Lysis Buffer.

RESULTS

Marker	Clone 1	Clone 2	Clone 3
CD45	2D1	HI30	MEM-28
CD3	SK7	UCHT1	OKT3/HIT3a
CD8	SK1	HIT8a	RPA-T8
CD4	SK3	OKT4	RPA-T4
CD16	B73.1	CB16	3G88
CD19	SJ25C1	HIB19	4G7
CD56	NCAM 16.2	MEM-188	MY-31
CD14	M5E2	61D3	HCD14

Table 1. Antibody clone compatibility Note that clones 2 and 3 for all markers were tested using PE. Each clone showed excellent separation between the positive and negative populations with the exception of OKT3 and HIT3a for CD3. We would suggest using either SK7 or UCHT1 clones for CD3 staining.

RESULTS

Lot-to-Lot Reproducibility Assessment

Population	Lyophilized PBMCs Lots	Cryopreserved PBMCs Lots	TruCytes™ Lymphocyte Subset Lots
Grans CD45+	NA	NA	2.5
Monos CD45+	13.9	34.5	4.4
Lymphs CD45+	10.2	15.2	0.6
T Cells CD3+	12.1	1.2	1.3
B Cells CD19+	36.6	19.8	5.7
NK Cells CD3-CD19-	29.1	8.8	1.6
T Helper CD4+	15.2	17.4	1.2
T Cytotoxic CD8+	18.7	18.7	2.3
NK Cells CD16+ CD56+	17.7	3.3	0.3
Classical Monos CD14+	1.6	8.3	0.1

Table 2. TruCytes™ Lymphocyte Subsets demonstrate less variation in % populations between lots than PBMCs. % CV was calculated for the percent population as evaluated on the Cytek Aurora across two lots of each different control material. Red indicates high variability. Blue indicates low variability.

Intra-Lot Sample-to-Sample Consistency

Population Type	Three replicates, tested on same instrument on same day	Same operator, on same instrument tested on 2 different days	Same operator, 2 different instruments on same day	2 different operators, on same instrument on same day
Grans CD45+	3.8	3.8	3.9	3.3
Monos CD45+	2.6	2.3	2.8	1.4
Lymphs CD45+	0.5	0.4	1.3	0.7
T Cells CD3+	0.2	0.4	0.2	0.3
B Cells CD19+	1.8	1.2	0.7	0.5
NK Cells CD3-CD19-	1.2	2.1	1.6	1.7
T Helper CD4+	0.3	0.3	0.4	0.3
T Cytotoxic CD8+	0.6	0.5	0.7	0.6
NK Cells CD16+ CD56+	0.3	0.4	0.6	0.2
Classical Monos CD14+	0.1	0.2	0.2	0.2

Table 3. TruCytes™ Lymphocyte Subset show intra-lot consistency. %CV was calculated for the percent population within the same lot of product to evaluate consistency between various users, instruments (Cytek Northern lights and Cytek Aurora), and time intervals.

Performance of TruCytes™ Lymphocyte Subset Control with Different Fixation Buffers

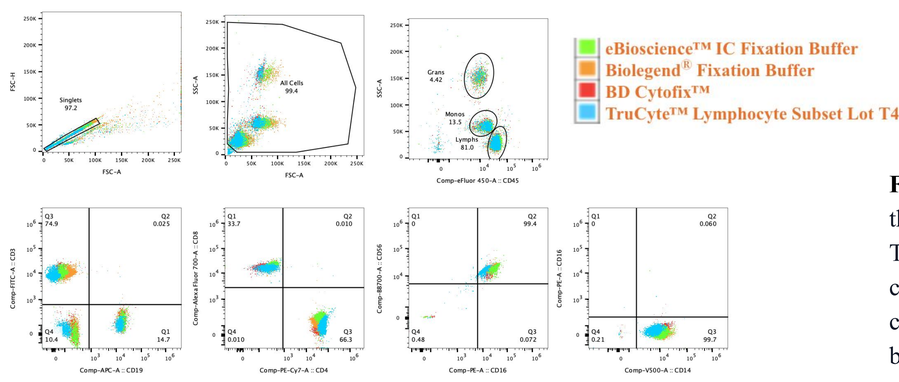


Figure 1. This study showcases the compatibility of the TruCytes™ Lymphocyte Subset control with common commercially available fixation buffers

Comparison of Performance using Lyse/Wash vs. Lyse/No Wash Protocols

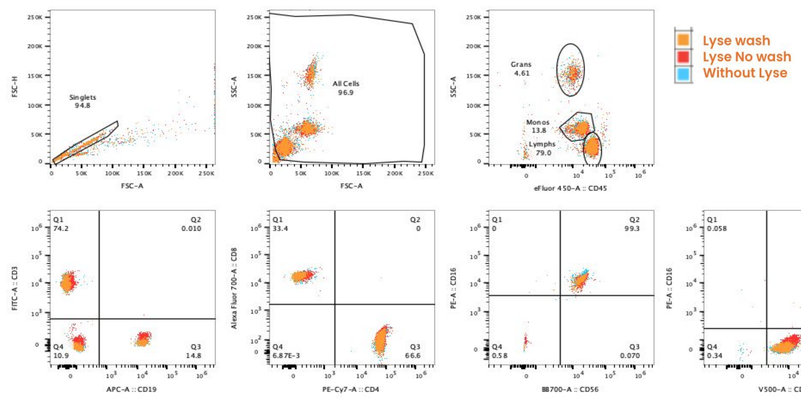


Figure 2. This study shows that TruCytes Lymphocyte Subset works in either lyse/wash or lyse/no wash protocols

CONCLUSIONS

TruCytes™ Lymphocyte Subset cell mimics can be easily adopted as a reliable, cost-effective alternative to PBMC-based controls in immunophenotyping assays as determined by:

- Compatibility with commonly used antibody clones.
- Consistency in lot-to-lot performance compared to PBMC-based immunophenotyping controls showing a range of 0.1% - 5.7% CVs versus 1.6% - 36.6% CVs for PBMC controls.
- Extended closed vial stability of 17 months when compared to the average stability of lyophilized PBMC-based immunophenotyping controls and equal to cryopreserved-based stability.
- Compatibility with existing workflows using common lyse and fixative buffers.