Enhancing Immunophenotyping Standardization in Clinical Labs with Quantitative BCMA or CD19 Cell Mimics

Zachary S. Clauss, Martina de Geus, Kanwal Palla

Slingshot Biosciences, Inc.

SLiNGSHOT

ABSTRACT

Immunophenotyping assays are vital in clinical laboratories for characterizing and quantifying cell surface antigens. This abstract explores the use of quantitative BCMA or CD19 cell mimics to enhance immunophenotyping practices. These cell mimics serve as reliable quantitative controls with known antigen density of BCMA or CD19 molecules. Incorporating the cell mimics as reference material enables standardization, increasing comparability and data consistency across laboratories. These mimics play a vital role for assay transfer, facilitating reliable data exchange between different research settings. Additionally, BCMA or CD19 cell mimics aid in receptor occupancy assessment method development and validation, optimizing staining conditions, antibodyconcentrations, and assay sensitivity and specificity.

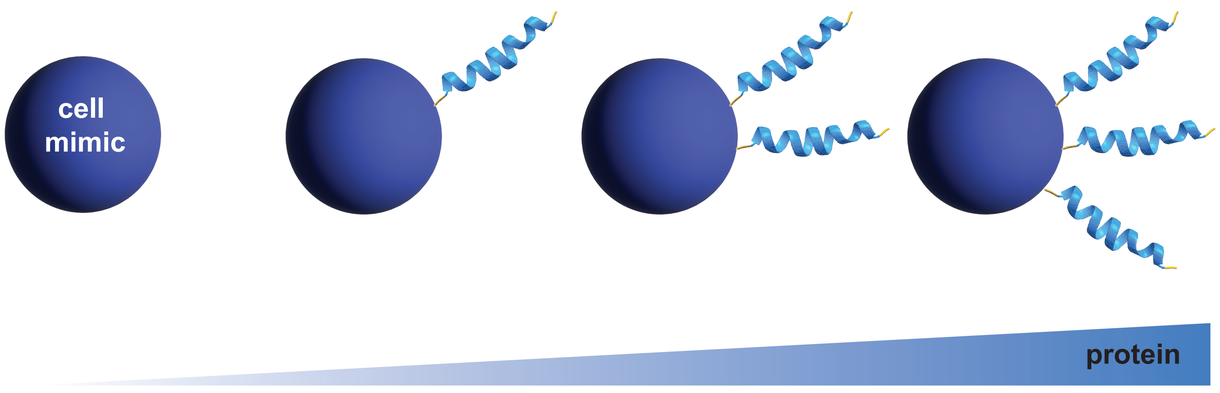
These cell mimics were designed using Slingshot Biosciences novel synthetic cell printing technology on a scalable platform. This innovative approach enables the design of cell mimics that are independently tuned along optical and biochemical parameters. By precisely controlling these parameters, our synthetic cell printing technology provides a versatile and customizable tool for immunophenotyping applications.

These quantitative cell mimics with known antigen density are a non-biohazardous and shelf stable alternative to primary cells. They require no maintenance prior to staining, enabling labs with limited resources to achieve reliable results. In summary, the utilization of quantitative BCMA or CD19 cell mimics enhances immunophenotyping practices, providing quality control, standardization, method validation, and cost-effectiveness. By incorporating this quantitative cell mimic, clinical labs can achieve consistent and reliable results while optimizing resource utilization and advancing immunophenotyping techniques.

INTRODUCTION

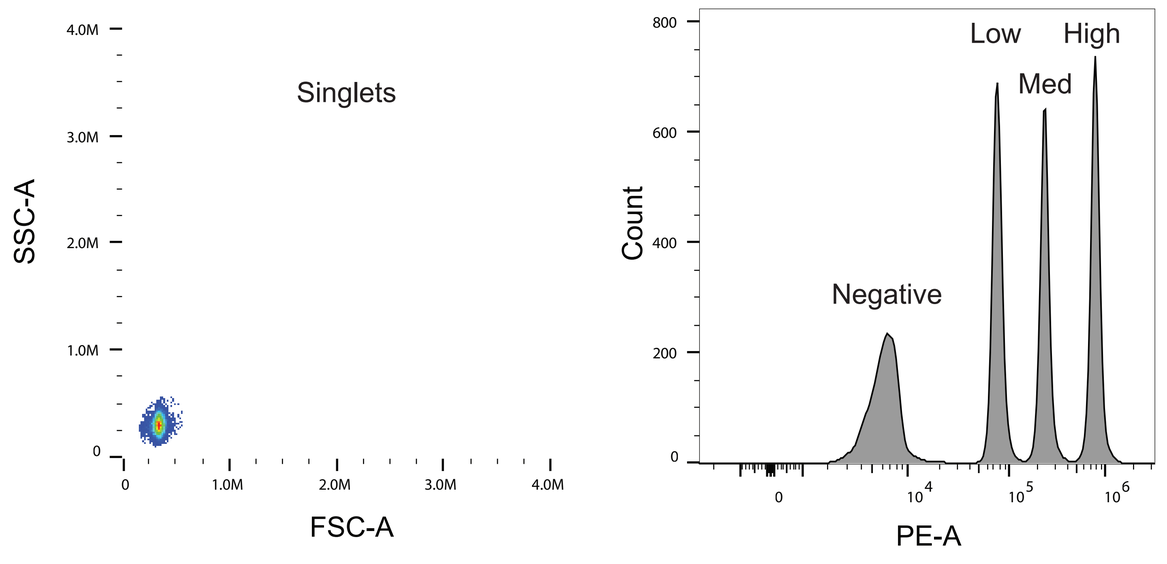
Approved CAR T cell therapies are, so far, only targeted to the proteins BCMA or CD19. Drug discovery laboratories work toward quantifying cell surface antigens during treatment. Alignment across laboratories requires validated standards to facilitate consistency and data comparison. Here, we demonstrate the versatility of quantitative BCMA and CD19 cell mimics to help standardize a primarily qualitative field.

METHODOLOGY



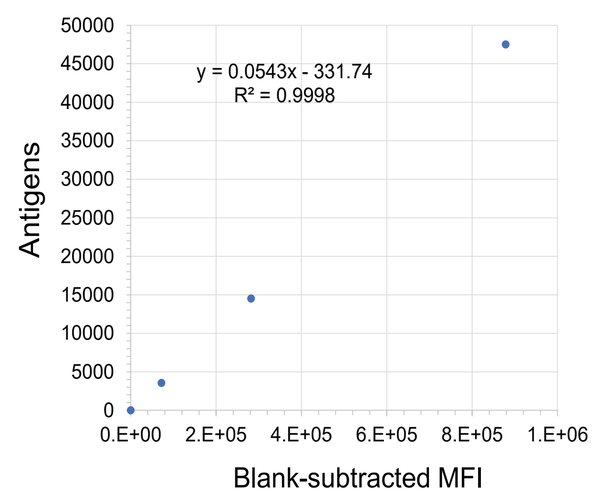
- Attach different levels of either BCMA or CD19 to our proprietary cell mimics
- Mix three distinct populations with an additional blank population
- Analyze with a variety of fluorophore/antibody conjugates on Cytek Aurora flow cytometer
- Report antigens per cell mimic (antigen density) for customer use as calibration curve

RESULTS



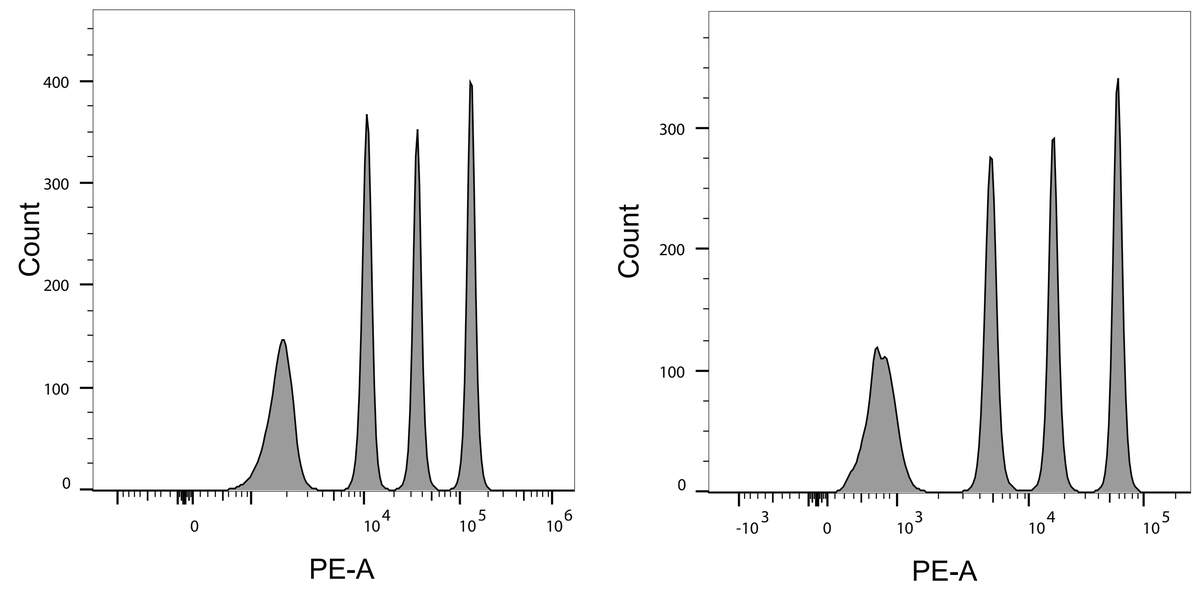
Representative data of the mixed four population BCMA quantitative antigen density cell mimic. Left: Scatter plot (SSC-A versus FSC-A) with gating on the target population. Right: Histogram in the YG1-A channel of the cell mimics stained with clone 19F2 conjugated to the fluorophore PE. Product format consists of a negative, low, medium, and high BCMA population. Data collected on Cytek Aurora.

RESULTS

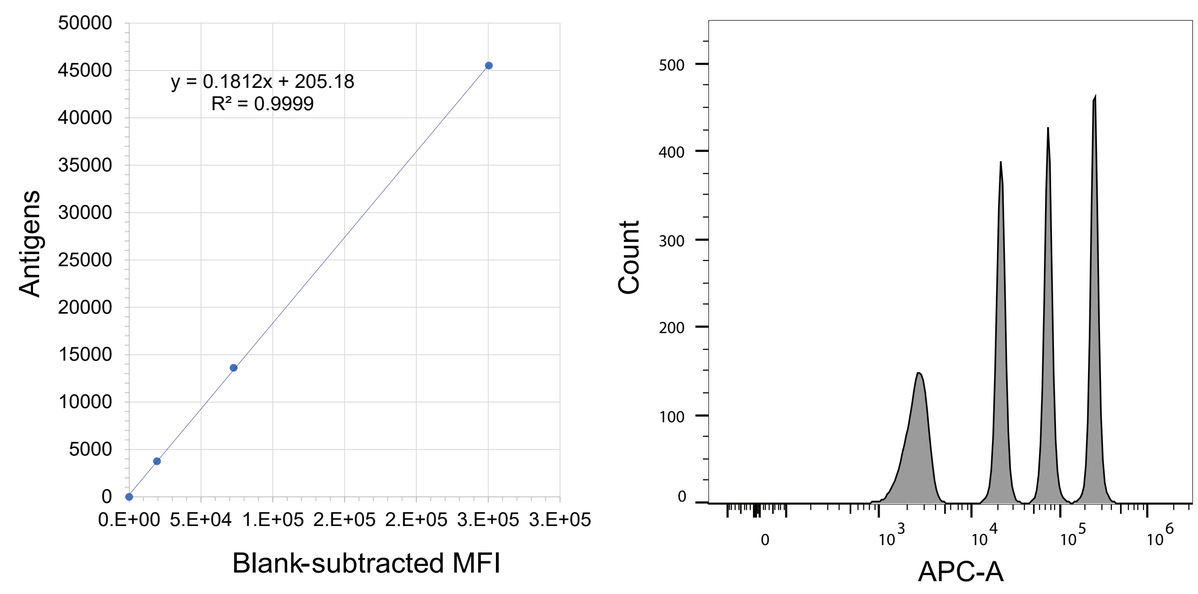


Lot	Average no. of antigens: low peak	Average no. of antigens: mid peak	Average no. of antigens: high peak
A	3532	11990	42752
B	3556	14522	47524
C	3755	13627	45521
Mean	**3614**	**13380**	**45266**
%CV	3.4	9.6	5.3

Left: Representative standard curve of antigens versus blank-substracted MFI. Stained with PE-19F2. Right: Inter-lot mean and coefficient of variation of each peak's determined antigens. Data collected on Cytek Aurora.



BCMA quantitative antigen density analyzed on traditional flow cytometers. Left: Cytoflex S. Right: BD Lyric. Stained with PE-19F2.



Left: Representative standard curve of antigens versus blank-substracted MFI. Stained with APC-19F2. Right: Histogram of APC-19F2 stained product used to create the standard curve. Data collected on Cytek Aurora.

Interlot cell line BCMA antigen densities

Cell Line	Lot A	Lot B	Lot C	Mean	%CV
MM.1S	4001	4197	4135	4111	2.4
H929	25561	26347	26137	26015	1.6

Cells and three different lots of BCMA quantitative antigen density cell mimics were stained with PE-19F2. Cell data was analyzed with each of the three lots to yield the reported antigen densities.

APC vs. PE staining

Cell Line	Lot C: PE	Lot C: APC
MM.1S	4135	4252
H929	26137	26275

Cell line staining with different fluorophores conjugated to clone 19F2 show nearly identical BCMA antigen density values.

CONCLUSION

Robust, multi-level BCMA quantitative antigen density cell mimic has been successfully developed. Intra-lot CVs of antigen numbers were <4.3%. Inter-lot CVs of antigen numbers were <9.6%. Population percentages of each peak varied minimally as well with equal proportions in all three lots. This products serves as a template for on-going development of a CD19 quantitative cell mimic.