

Enhancing Immunophenotyping Standardization with Quantitative BCMA or CD19 Decorated Hydrogels



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ABSTRACT

Hydrogel-based cell mimics with known amounts of antibody-accessible BCMA or CD19 molecules serve as quantitative controls for readily measuring the amount of protein in biological samples. Cell mimics with multi-level antigen expression (~45000, ~13000, ~3500 antigens per particle) can be used to generate a standard curve of antigen counts and calculate the antigen density of an unknown sample. Consistent antigen densities were observed across multiple cell lines for BCMA (CV<2.44%) and CD19 (CV<4.03%) when evaluated against three lots of TruCytes™ BCMA Quant or CD19 Quant cell mimics.

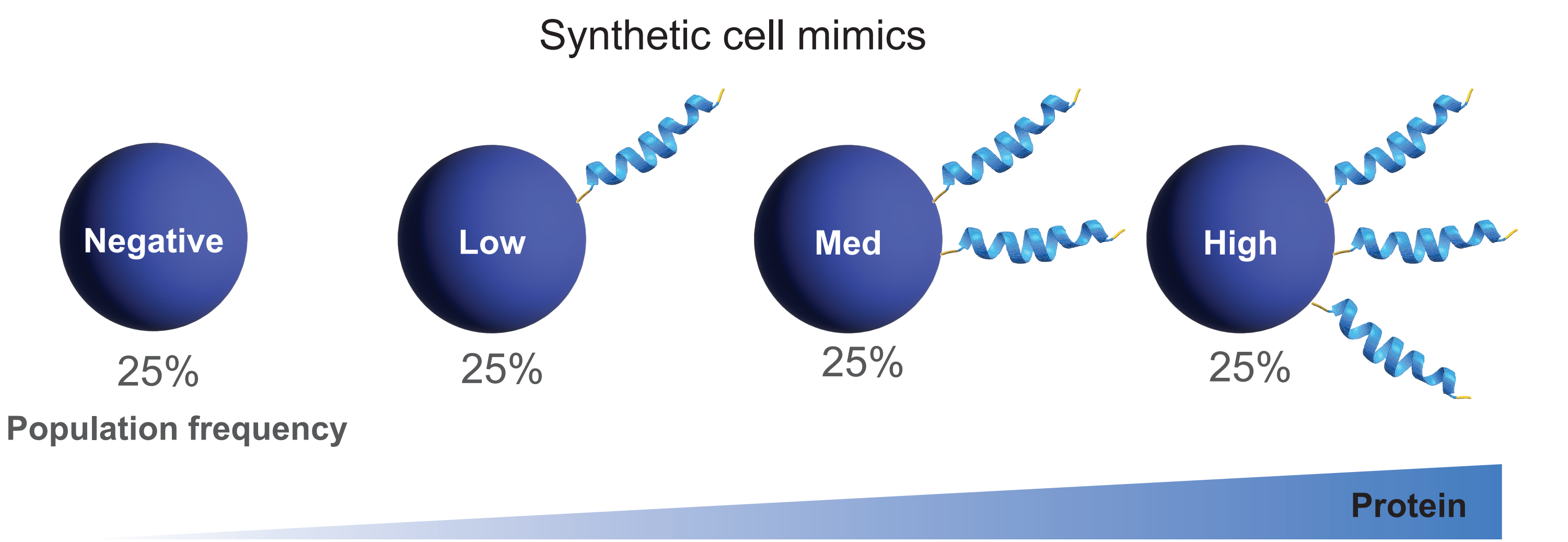
INTRODUCTION

Immunophenotyping assays are ideal for characterizing biological samples for cell subset identification. In cell therapy, quantitating the amount of target protein on patient samples can inform dosage and improve supply challenges of manufactured cell therapy products.

Quantitation via flow cytometry poses additional challenges given the number of variables associated with the instrument, reagent, and sample preparation. Unique quantitative standards have been developed that address these issues. Here we present our novel approach to quantitation by flow cytometry, using a hydrogel based particle with known numbers of antibody-accessible BCMA or CD19 molecules. This hydrogel contains the extracellular domain of the antigen present on the cell, whereas other quantitative methods rely on indirect Fc-based binding or MESF correlation via hard-dyed beads. Slingshot’s TruCytes™ BCMA/CD19 Quant control can be stained with the same fluorescently labeled antibody used for staining biological cells, eliminating the need to know the antibody F/P ratio when calculating antigen density.

These quantitative cell mimics were designed using Slingshot Bioscience’s 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 technology provides a versatile and customizable tool for flow cytometry-based immunophenotyping applications.

METHODOLOGY



- Attach different levels of either BCMA or CD19 to hydrogel cell mimics
- Mix four distinct populations in equal parts, one negative and three positive with increasing levels of protein (i.e. ~45000, ~13000, ~3500 BCMA antibody accessible antigens per particle)
- Analyze with an antibody/fluorophore conjugate on the Cytex Aurora flow cytometer
- Create a standard curve using reported antibody accessible antigen per cell mimic (antigen density)
- Use standard curve to determine unknown antigen density of biological sample of interest

RESULTS

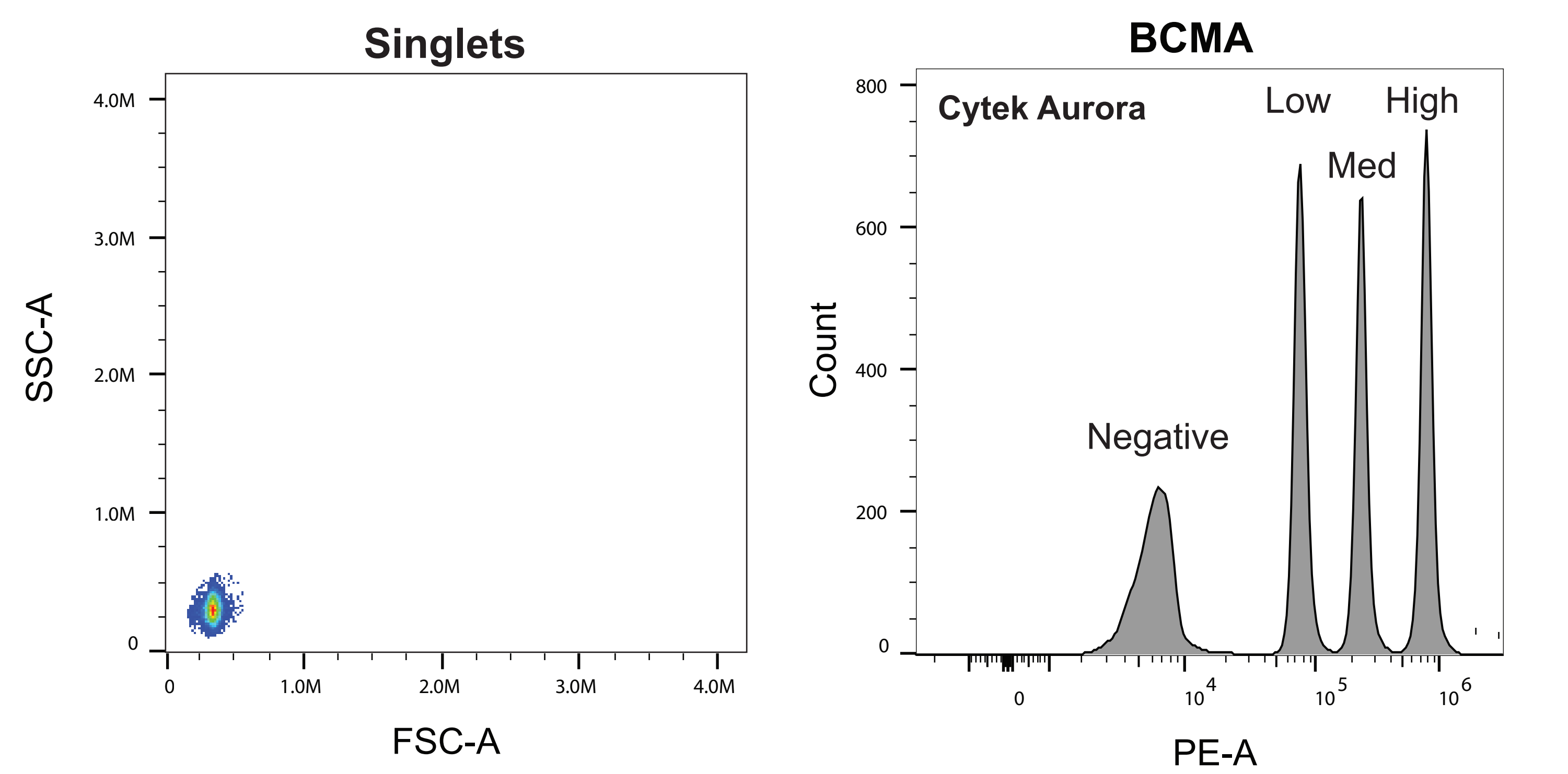
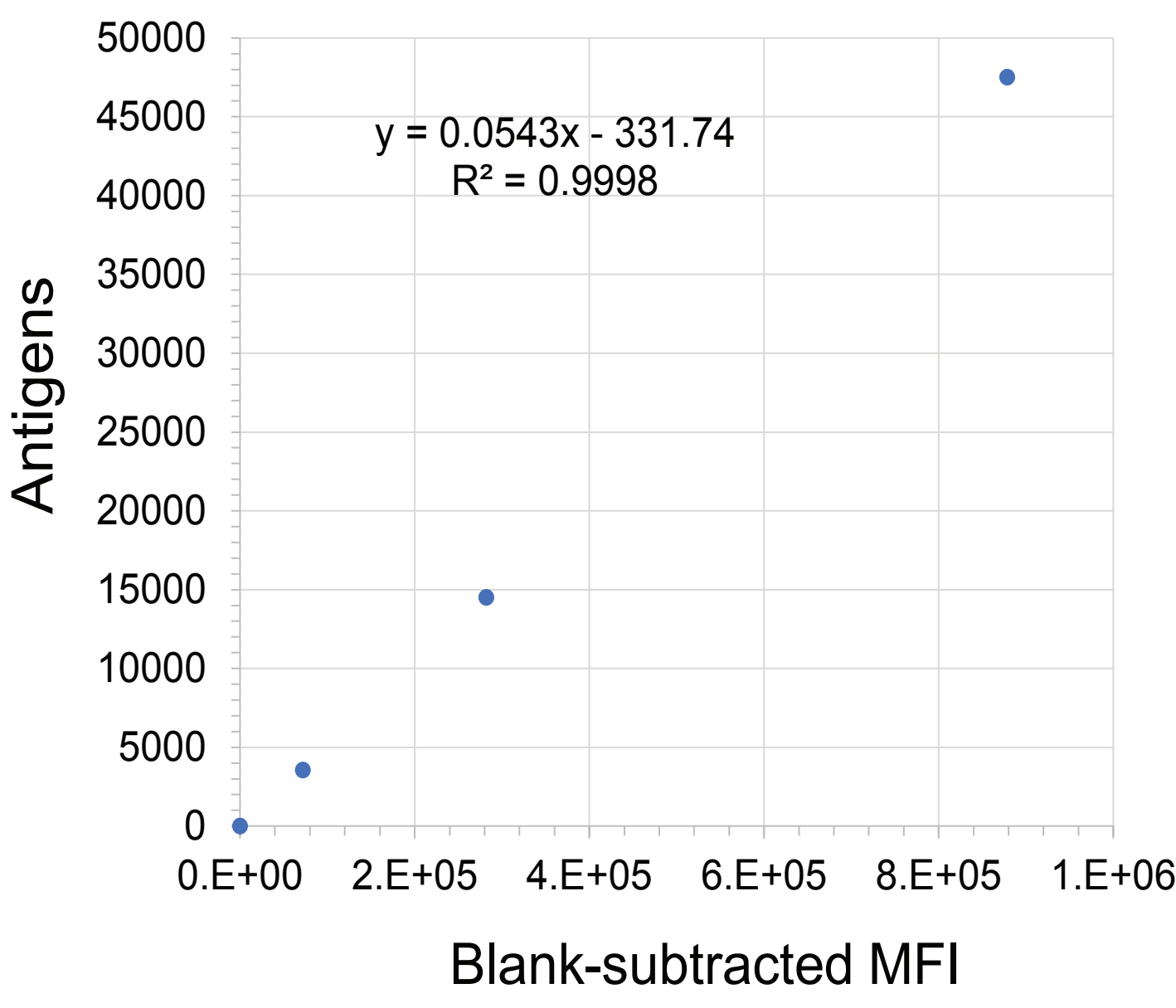


Figure 1. Representative data of the four mixed populations (negative, low, medium, high) of TruCytes BCMA Quant. Left: Scatter plot (SSC-A versus FSC-A) with gating on the target population. Right: Histogram in the YG1-A channel of the cell mimics stained with clone 19F2 conjugated to the fluorophore PE. Data collected on Cytex Aurora.

RESULTS



	Low peak	Med peak	High peak
No. BCMA antigens	3556	14522	47524

Figure 2. Representative standard curve of blank-subtracted MFI versus reported number of antibody accessible BCMA antigens for each population. TruCytes BCMA Quant was stained with PE-19F2. Data collected on Cytex Aurora.

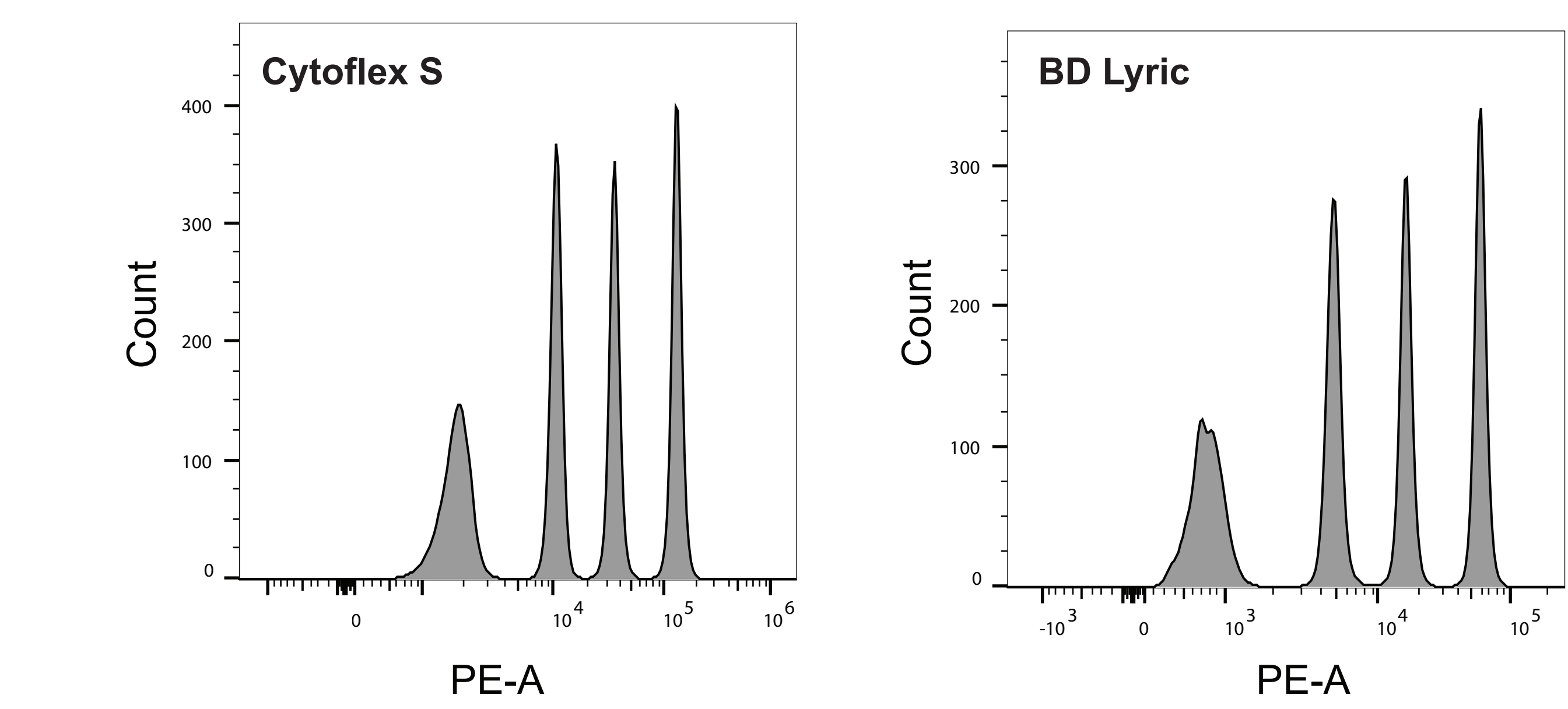
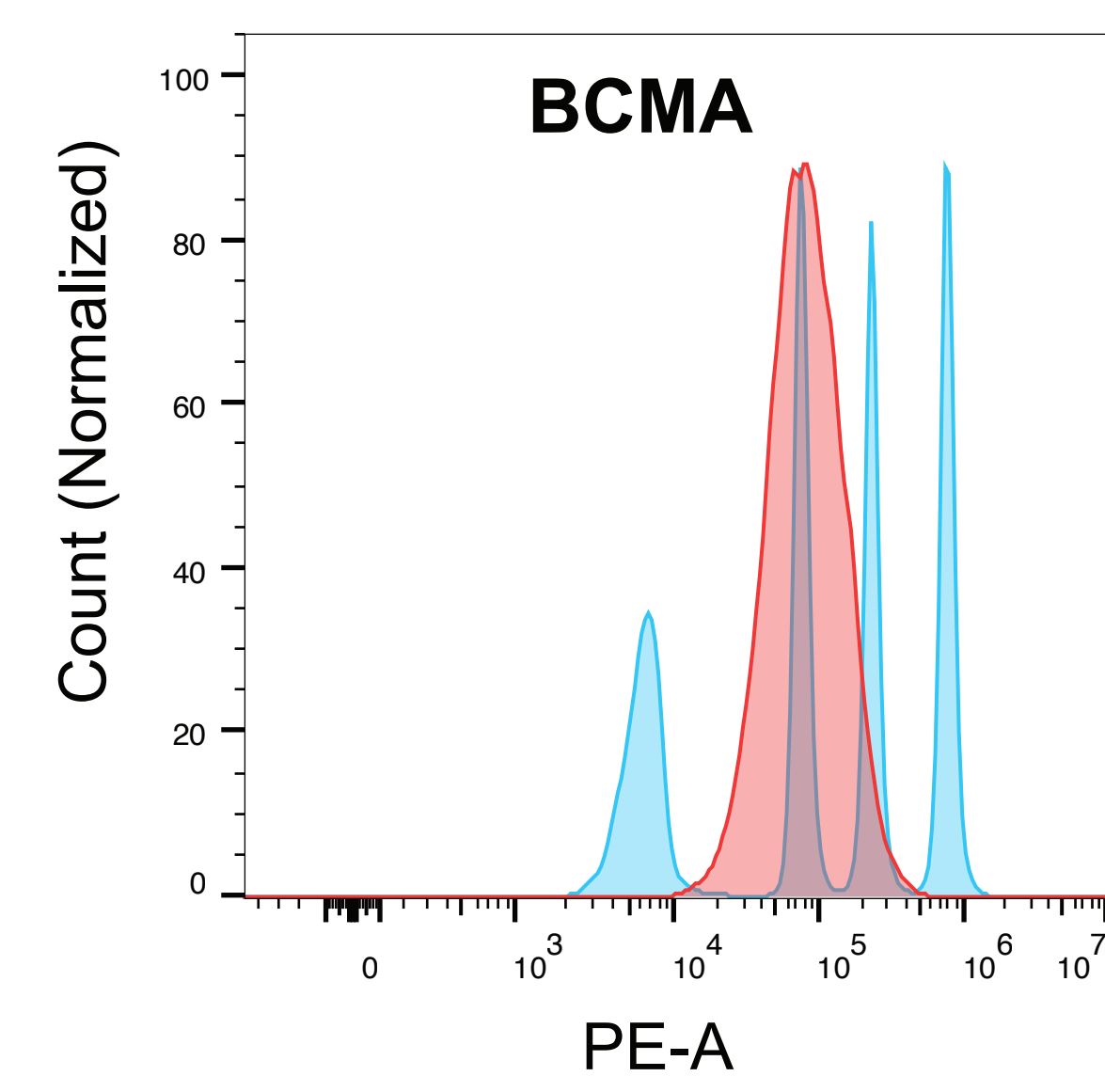
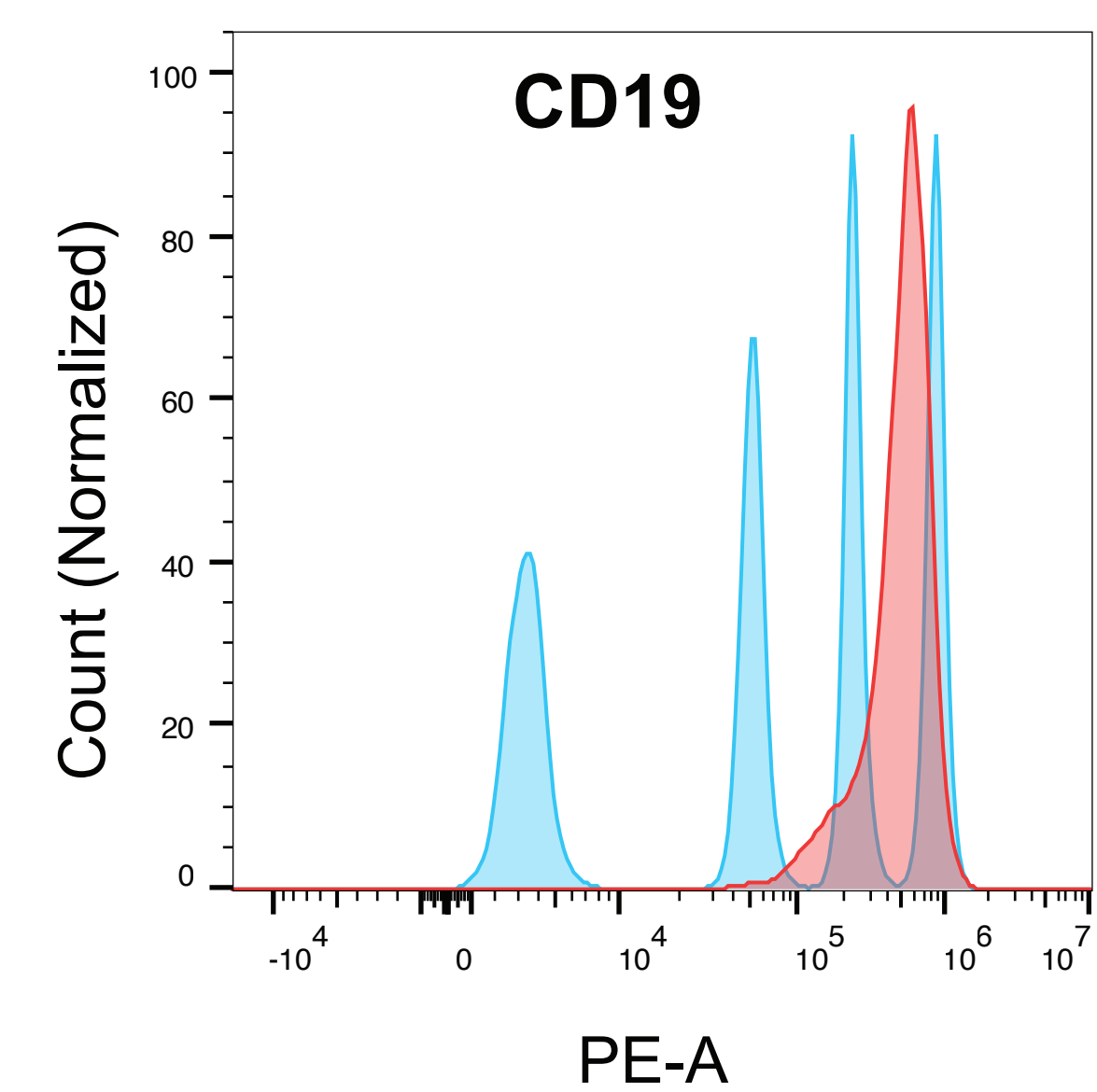


Figure 3. BCMA quantitative antigen density analyzed on conventional flow cytometers. Left: Cytex S. Right: BD Lyric. TruCytes BCMA Quant was stained with PE-19F2.



BCMA expression levels quantitated by 3 lots of TruCytes BCMA Quant					
Cell line	Lot A	Lot B	Lot C	Mean	%CV
MM.1S	4001	4197	4135	4111	2.44
H929	25561	26347	26137	26015	1.56

Figure 4. Right: BCMA antigen density on two cell lines (MM.1S ad H929) using three different lots of TruCytes BCMA Quant and their reported antibody accessible antigen counts. Biological cells and cell mimics were stained with PE-19F2. Left: Representative histogram of the YG1-A channel of MM.1S (red) and TruCyte BCMA Quant (blue). Data collected on Cytex Aurora.



CD19 expression levels quantitated by 3 lots of TruCytes CD19 Quant					
Cell line	Lot A	Lot B	Lot C	Mean	%CV
Daudi	54298	53658	50398	52784	3.96
Nalm6	30731	30404	28501	29879	4.03
Raji	59981	59265	55678	58308	3.95

Figure 5. Right: CD19 antigen density on three B-cell lines (Daudi, Nalm6, Raji) using three different lots of TruCytes CD19 Quant and their reported antibody accessible antigen counts. Biological cells and cell mimics were stained with PE-HIB19. Left: Representative histogram in the YG1-A channel of Nalm6 (red) and TruCytes CD19 Quant (blue). Data collected on Cytex Aurora.

CONCLUSION

We successfully developed BCMA and CD19 quantitative cell mimics with known amounts of antigen. TruCytes™ BCMA/CD19 Quant controls enabled quantitation of BCMA and CD19 antigen density on biological cells - for BCMA on MM.1S and H929 (CV<2.44%) and CD19 on Daudi, Nalm6, and Raji (CV<4.03%), using three different lots. With minimal lot-to-lot variability, quantitative cell mimics can reliably be used to evaluate antigen density on biological samples, adding greater depth to flow cytometry-based immunophenotyping assays.