Advancing Flow Cytometry with a Novel Antigen Quantification Method

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Abstract

Flow cytometry assays play a key role in characterizing and quantifying cell surface antigens of interest throughout different phases of the cell therapy process. This poster takes a deeper dive into the linearity of quantitative BCMA and CD19 products offered through Slingshot Biosciences compared to other commercially available quantitation methods. Here we demonstrate the performance of Slingshot TruCytes™ BCMA and CD19 quantitative synthetic cells versus two different competitors across multiple instrument platforms. We also assess as linearity through a "cold titration" which uses both labeled and unlabeled BCMA and CD19 antibodies. Our results show that Slingshot products show lower or equivalent %CVs across instruments compared to the existing standards.

Introduction

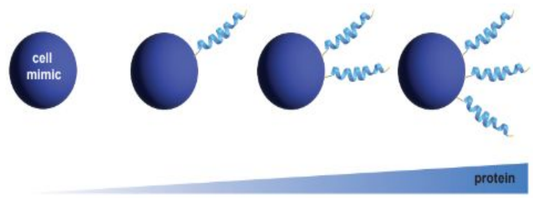
Flow cytometry is an essential part of any pharmaceutical or cell therapy workflow. It can provide crucial data throughout many parts of the life cycle of these programs, from research and drug discovery through clinical trials and beyond. However, variations in methods across different sites, between operators, reagents, and instruments can provide challenges with the use of this technology. This variation can carry over into antigen quantitation methods by flow cytometry.

The current standard flow cytometry quantitation method involves creating a standard curve which correlates signal intensity of a hard-dyed particle, such as PE, with known concentrations of dye (MESF based quantification). The antigen density of the unknown sample is obtained by inferring values from this standard curve as long as the degree of labeling for the fluorescent antibody is known. Other methods available use capture antibodies to generate a standard curve rather than hard-dyed particles.

Slingshot Biosciences offers a novel alternative, which is an epitope-specific standard for use in quantification through flow cytometry. So far, the commercially approved CAR-T cell therapies on the market are made to target BCMA or CD19, which emphasize the need for epitope-specific quantitative controls for flow cytometry testing in the cell therapy landscape. Slingshot's quantitative synthetic cells measure the antibody accessible antigens on the surface; providing a more direct measurement and removing the need to know your antibody's degree of labeling when calculating results. Quantitation of these targets before, during, and after completion of therapy plays a key role in evaluating the efficacy of the cell therapy treatment.

METHODOLOGY

BCMA/CD19 Quantitative Antigen Density Control Design

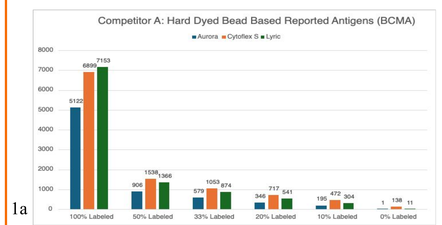


Representative diagram of how Slingshot TruCytes™ BCMA and CD19 quantitative synthetic cells are conjugated with known amounts of antigen to derive a calibration curve for antigen quantification

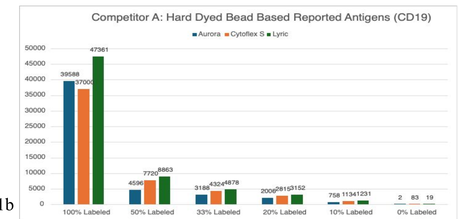
% of Labeled Antibody	Amount of Labeled	Amount of Unlabeled
100% Labeled	5 uL	0 uL
50% Labeled	2.5 uL	2.5 uL
33% Labeled	1.67 uL	3.37 uL
20% Labeled	1 uL	4 uL
10% Labeled	0.5 uL	4.5 uL
0% Labeled	0 uL	5 uL

A "cold titration" was performed by staining 400,000 Raji (CD19+) and MM1.S (BCMA+) cells with different ratios of labeled and unlabeled CD19 or BCMA antibody to evaluate the linearity of quantification. Unlabeled antibody was used to ensure that saturation of the available binding sites was achieved. The table above is representative of the different ratios tested. Note: each standard was stained with 5 uL of labeled antibody. All samples were stained in 100 uL total volume.

RESULTS



1a



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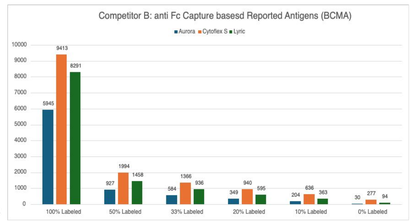

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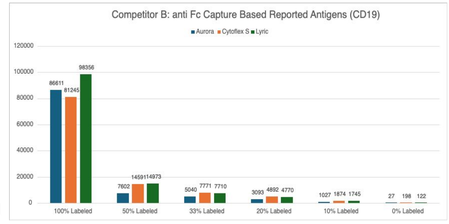

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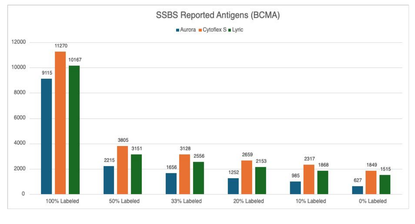

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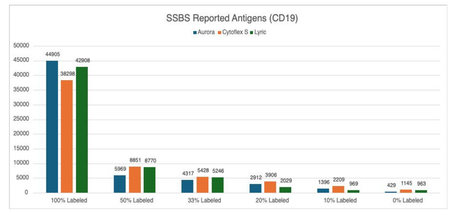

1f

Figure 1a-f: Comparison of reported antigens across three different instrument platforms (Cytek Aurora, Beckman Coulter Cytoflex S, and BD FACSLyric) for the three different antigen quantitation methods (Competitor A: hard-dyed beas, Competitor B: anti-Fc capture, and Slingshot Biosciences) for BCMA and CD19 respectively. Raji cell line was used for CD19 quantitation while MM1.S cell line was used for BCMA quantitation.

Figure 2: %CV Across Instruments

	Hard Dyed Bead	anti-Fc Capture	SSBS
BCMA	17.32	22.45	10.58
CD19	13.05	9.86	8.06

Figure 2: Comparison of %CV across the three different instruments tested (Cytek Aurora, Beckman Coulter Cytoflex S, and BD FACSLyric) for each quantitation method. This was performed only for the 100% labeled antibody point since the others titration points were found not to have performed linearly.

Figure 3a: Summary of Theoretical Antigen Density and R^2 for BCMA

	Aurora			Cytoflex S			Lyric		
	Hard Dyed Bead	anti-Fc Capture	SSBS	Hard Dyed Bead	anti-Fc Capture	SSBS	Hard Dyed Bead	anti-Fc Capture	SSBS
100% Labeled	5122	5945	9115	6899	9413	11270	7153	9413	10167
50% Labeled	2501	2705	4870	3446	4504	6463	3582	4504	5835
33% Labeled	1645	1707	3455	2296	2927	4861	2390	2927	4390
20% Labeled	970	955	2323	1376	1700	3579	1436	1700	3235
10% Labeled	474	435	1474	687	813	2618	719	813	2369
0% Labeled	0	0	0	0	0	0	0	0	0
R^2	0.89	0.90	0.86	0.90	0.91	0.84	0.89	0.90	0.82

Figure 3b: Summary of Theoretical Antigen Density and R^2 for CD19

	Aurora			Cytoflex S			Lyric		
	Hard Dyed Bead	anti-Fc Capture	SSBS	Hard Dyed Bead	anti-Fc Capture	SSBS	Hard Dyed Bead	anti-Fc Capture	SSBS
100% Labeled	39588	86611	44905	37000	81245	38298	47361	98356	42908
50% Labeled	19333	38438	22665	18479	37828	19680	23717	45028	21927
33% Labeled	12713	23899	15252	12311	24189	13473	15826	28510	14934
20% Labeled	7496	13133	9322	7380	13771	8508	9506	16030	9339
10% Labeled	3661	5829	4874	3686	6412	4785	4761	7339	5143
0% Labeled	0	0	0	0	0	0	0	0	0
R^2	0.86	0.87	0.85	0.90	0.91	0.89	0.89	0.90	0.89

Figure 3a&b: Summary of reported antigens across three different instrument platforms (Cytek Aurora, Beckman Coulter Cytoflex S, and BD FACSLyric) for the three different antigen quantitation methods (Competitor A: hard-dyed beas, Competitor B: anti-Fc capture, and Slingshot Biosciences) for BCMA and CD19 respectively. In addition, R^2 results were calculated to determine the strength of the linear relationship between theoretical and reported antigen densities as seen in figure 1.

DISCUSSION

When comparing the antigen density across instruments for each quantitation method, it can be seen that the %CV is lowest when using the Slingshot quantitative controls when assessing 100% labeled BCMA and CD19 antibody (Figure 1a-f and Figure 2). These findings while expected due to previous studies, did not repeat the same significantly lower %CV. (1) In addition due to the inability to establish linearity, as discussed below, the other titration points were unable to be evaluated in regards to %CV across instrument platforms. As outlined below, further studies are required.

Based on the R^2 results (Figure 3a&b) we were unable to prove a strong linear relationship between CD19/BCMA and either of the three different quantitative products tested using a "cold titration". The use of both labeled and unlabeled antibodies was intended to ensure that the antibody binding sites would be saturated on the cells of interest. This allowed us to determine a theoretical antigen density based on how we would expect the results to change as the amount of labeled antibody was decreased. Unfortunately, when comparing the theoretical and reported antigen densities, it can be seen that the linear relationship is not very reliable.

One hypothesis is that the kinetics of the large PE molecule caused preferential binding of the unlabeled CD19/BCMA antibodies rather than the PE conjugated antibodies. Steric hindrance is a well known phenomena in which there is a reduction or loss of detectable fluorescence because one antibody impedes the binding of another to the target on an antigen when the antibodies are used together. (2) The drastic decrease in reported antigen count at the second (1:1) and subsequent titration points versus what was expected based on the theoretical antigen density suggest that this would be a plausible explanation for the findings.

To further investigate the theory of steric hindrance, additional studies will be performed using a smaller molecule such as FITC with the same "cold titration" format as was performed with PE. Furthermore, a standard titration will be performed by adding different amounts of labeled antibody to the cells of interest, excluding the unlabeled antibody. We feel this will help to answer the question of what exactly contributed to the outcomes seen in this study and to help investigate linearity amongst different antigen quantitation methodologies. These studies will also be performed across the same three instruments to further evaluate the %CV of the different quantification methods across different instrument platforms.

CITATIONS

1. **Dankar, A, et al. Developing linearity and quantification workflows for flow cytometry with synthetic hydrogel-based particles. Poster presented at: Cell Therapy Analytical Development Summit, December 4-6 2023; Boston, MA.**
2. **Matos, D.M. (2021), Steric hindrance: A practical (and frequently forgotten) problem in flow cytometry. Cytometry, 100: 397-401. https://doi.org/10.1002/cyto.b.21959**

ACKNOWLEDGEMENTS

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