Development of a Dried-down, Multicolor Reagent Solution for Enhanced Flow-cytometric Applications

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Abstract

Multicolor flow cytometry provides a powerful tool to identify, analyze and enumerate multiple cell populations phenotypically, thereby, making it a critical tool for hematological testing and diagnosis of hematological malignancies, as well as immune monitoring. As part of its custom reagents program, BD offers panel design tools and manufacturing of dried-down reagent cocktails for a diverse range of flow cytometry research applications. The dried-down cocktails afford enhanced reagent stability, simplify the assay workflow and enable assay standardization across instruments, operators and testing sites.

The emergence of high-performing BD Horizon Brilliant™ dyes has resulted in significant demand to utilize these dyes in multicolor reagent panels. Conjugates made with BD Horizon Brilliant™ dyes are bright and provide excellent performance across multiple assays and applications. However, cocktailing multiple BD Horizon Brilliant™ reagents may result in unwanted dye-to-dye interactions potentially impacting the stability of the cocktails. To address this issue, BD has developed a technology that enables the delivery of multiple BD Horizon Brilliant™ reagents in a single-use, dried-down format. The development of this technology enables BD to manufacture dried-down reagent cocktails containing up to five BD Horizon Brilliant™ reagents.

To demonstrate feasibility of this technology, we designed 2 panels (5-color and 7-color) comprising CD3, CD4, CD45RA, CD25, CD127, CD15s and CD161 for identification of regulatory T cells (Tregs) and characterizing the different Treg subsets (naïve, effector and transitional), as well as the IL-17-producing Tregs (CD161$^+$) and the potently immunosuppressive CD15s$^+$ Tregs. As part of assessing the feasibility of the reagent drying technology, we compared the performance of the 7-color Treg panel in a dried-down state and as a liquid cocktail using the BD FACSLyric™ cell analyzer. Our results show that the performance of the dried-down cocktail is free of unwanted dye-to-dye interactions and is equivalent to that of the liquid cocktail in terms of resolution of the different functional Treg subsets. Specifically, the percent of positive cells measured for the different Treg subsets (in a given sample) are comparable between the dried-down and the liquid cocktail. This data demonstrates the feasibility of the reagent drying technology.

Technology Overview

BD custom multicolor panels – BD Horizon™ Dri Chroma multicolor cocktails offer:

- **Greater workflow efficiency**
  - Proprietary technology to dry down up to 5 BD Horizon Brilliant™ dyes in one tube reduces the need for manual pipetting steps in your laboratory
  - Improves ease-of-use by providing up to 14 parameters in a single tube format
  - Reduces time to results with a streamlined workflow: simply resuspend the dried reagents, then add your sample, with no need for cocktailing
- **Greater standardization**
  - All-in-one tube format reduces operator errors due to liquid pipetting and cocktailing
  - Batch manufacturing of dried panels provides ideal format for inter- and intra-laboratory assays, enabling better data reproducibility
  - Reduces day-to-day variability, resulting in more consistent results
- **Greater stability**
  - 1-year shelf life when stored at room temperature (20-25°C)
  - Pouched in airtight, resealable, light-resistant foil bags
  - Resuspension of dried reagents with BD Hoizon™ Brilliant Stain Buffer or BD Horizon™ Brilliant Stain Buffer Plus ensures minimal dye-to-dye interaction during staining
- **Greater flexibility**
  - Evaluation tubes included with every order ensure panel performance before scale-up
  - Dedicated BD Custom Technology Team available to optimize panel design for research use only (RUO) applications
  - Custom packaging options available to meet your lab's specialized needs

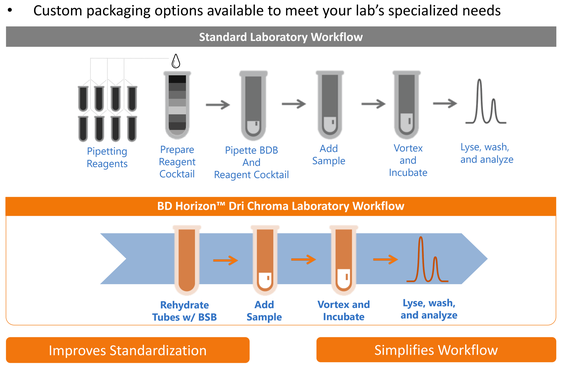
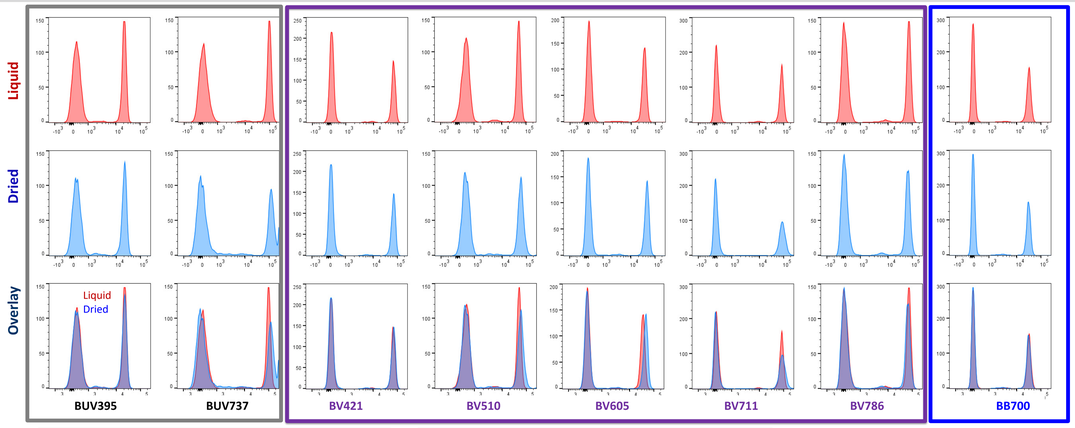


Figure 1. Offering a dried reagent cocktail in single tube maximizes lab efficiency by eliminating repetitive pipetting and extending the shelf life of liquid cocktails. A single tube format allows for standardization across instruments by eliminating variation due to reagents.

Results 1. Single Color Analysis for CD4$^+$



Fluorochrome	Sample	% Positive	MFI*
BUV395	Liquid	39.8	20,879
	Dried	39.2	20,652
BUV737	Liquid	39.4	96,541
	Dried	39.2	95,024
BV421	Liquid	37.6	64,739
	Dried	39.3	62,917
BV510	Liquid	38.8	47,917
	Dried	39.6	56,941

Fluorochrome	Sample	% Positive	MFI*
BV605	Liquid	40.4	33,194
	Dried	40.2	44,404
BV711	Liquid	40.7	81,030
	Dried	38.8	87,046
BV786	Liquid	40.5	70,130
	Dried	37.7	63,226
BB700	Liquid	38.2	30,325
	Dried	38.1	29,209

Figure 2. Representative histogram analysis of single color CD4 conjugates across the violet, ultraviolet, and blue laser lines. Overlay plots demonstrate that the drying of the CD4 conjugate (in different colors) does not impact its performance.

*MFI=Median Fluorescence Intensity

Results 2. Evaluation of the 5-color Treg Panel

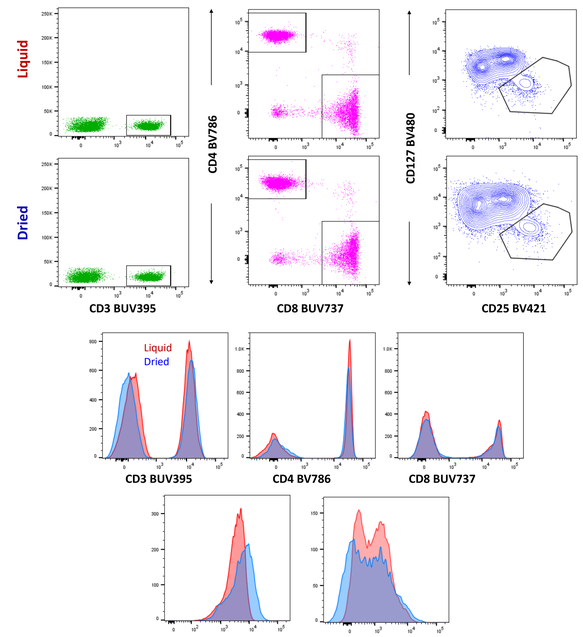
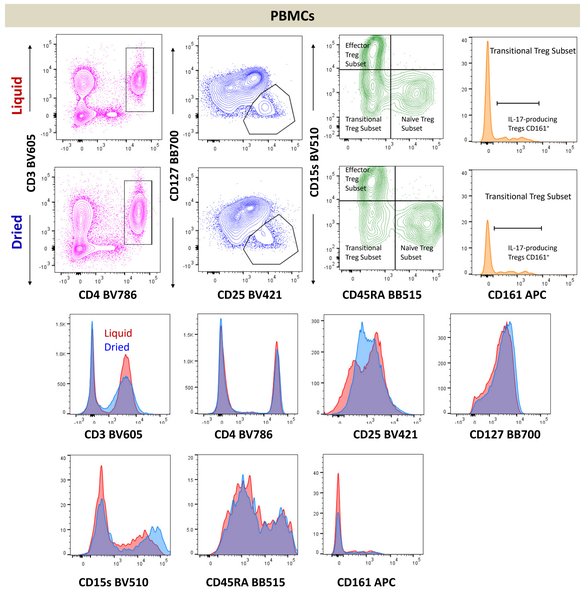


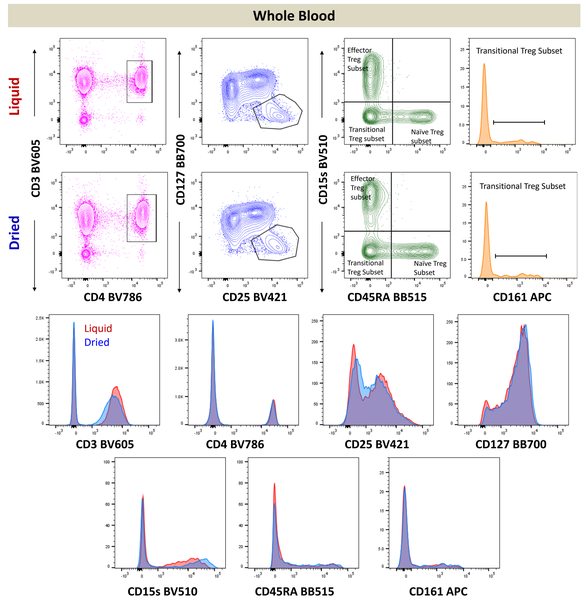
Figure 3. A 5-color panel for the identification of Tregs (CD3$^+$CD4$^+$CD127^{low}CD25^{high}) was used to compare the impact of drying on the biological resolution of Tregs. Samples were stained using lysed whole blood from a healthy donor using BD Horizon™ Brilliant Stain Buffer (Cat. No. 659611) and the antibodies listed in the panel. Samples were analyzed on a BD LSRFortessa™ X-20 flow cytometer and data analysis was performed using BD FACSDiva™ and FlowJo™ software.

Conjugate	Sample	% Positive	MFI*
CD3 BUV395	Liquid	42.5	11834
	Dried	43.6	13673
CD4 BV786	Liquid	58.2	33107
	Dried	60.2	30646
CD8 BUV737	Liquid	34.2	32124
	Dried	32.8	31208
CD25 BV421	Liquid	7.2	5453
	Dried	7.0	7218
CD127 BV480	Liquid	92.6	3704
	Dried	92.2	6176

*MFI=Median Fluorescence Intensity

Results 3. Evaluation of the 7-color Treg Panel





Conjugate	Sample	PBMCs % Positive	PBMCs MFI*	Whole Blood % Positive	Whole Blood MFI*
CD3 BV605	Liquid	23.7	3198	63.6	5734
	Dried	23.8	2511	62.8	4486
CD4 BV786	Liquid	41.2	32590	39.9	26792
	Dried	39.9	34635	39.3	25317
CD127 BB700	Liquid	91.3	2974	89.2	3802
	Dried	91.1	3693	89.9	4233
CD25 BV421	Liquid	7.0	7974	10.5	12024
	Dried	6.2	10346	9.9	11703

Conjugate	Sample	PBMCs % Positive	PBMCs MFI*	Whole Blood % Positive	Whole Blood MFI*
CD45RA BB515	Liquid	20.1	1165	26.1	7301
	Dried	17.8	1202	26.8	8642
CD15s BV510	Liquid	7.1	10731	53.1	9552
	Dried	10.8	35393	51.7	26664
CD161 APC	Liquid	20.1	1165	22.7	1502
	Dried	17.8	1202	27	1465

*MFI=Median Fluorescence Intensity

Figure 4. Biological resolution of Tregs in PBMCs and whole blood using a 7-color panel. Samples were acquired on a 12-color BD FACSLyric™ cell analyzer and data analysis was performed using FlowJo™ software. Population statistics are shown as percent of parent population for the respective subsets. There is minimal impact in the resolution and population percentages of major Treg subsets upon drying.

Conclusions

- The technology for BD Horizon™ Dri Chroma reagents provides a powerful tool to simplify and standardize laboratory workflow and maximize lab efficiency by eliminating repetitive pipetting and human-prone errors. The dried cocktail will deliver easy-to-use standardization of multicolor flow cytometry data across multiple instruments and set the stage for its application in diverse areas of flow cytometry-based research.
- The single-color CD4 conjugates reported here across the violet, ultraviolet, and blue laser lines demonstrate comparable performance between the dried and liquid reagents with minimal impact on reagent brightness.
- A 5-color Treg panel containing five BD Horizon Brilliant™ reagents has been dried down and proven to be comparable to the liquid panel with no significant impact on the resolution of the Treg populations.
- To further demonstrate the feasibility of the drying technology, a 7-color Treg panel enables the characterization of the Treg population and its subsets. Results from the panel showed the resolution for both dried-down and liquid cocktails are comparable by means of percent population across the respective Treg subsets.