Dried Antibody Cocktails: Setting the Benchmark for Fast and Robust Flow Cytometric Analysis in Cell and Gene Therapy

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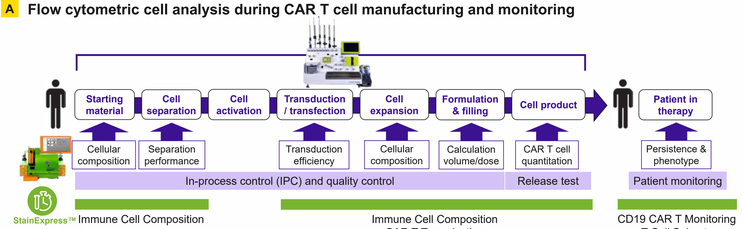
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Introduction

Flow cytometry is a key technology in the development, manufacturing, and quality assessment of cell and gene therapy (CGT) products. Notably within the CGT landscape is CAR T cell therapy, a major breakthrough in the treatment of hematological malignancies, for which flow cytometric analysis has become an indispensable tool. For the clinical manufacturing of CAR T cells as well as for research and pre-clinical applications, the specific detection of CAR expression along with the characterization of the cellular composition is of high importance (Figure 1a). In addition, monitoring patients is crucial for assessing therapy effectiveness and adjusting the treatment. Ensuring comparability across treatment centers worldwide is vital for consistent patient care. However, numerous manual steps when using liquid antibodies contribute to analytical variability and introduce operator-dependent errors. To overcome this, we developed dried antibody cocktails that allow fast and robust flow cytometric cell analysis, combinable with highly specific CAR detection (Figure 1b).

Figure 1

A Flow cytometric cell analysis during CAR T cell manufacturing and monitoring



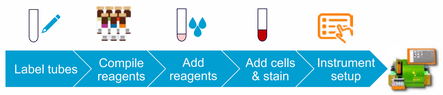
B StainExpress™ off-the-shelf portfolio

MQ 10 Filter	Immune Cell Composition	CAR T Transduction	CD19 CAR T Monitoring	T Cell Subsets
V1: 450/50 nm	CD45 VioBlue®	CD45 VioBlue®	CD8 VioBlue®	CD8 VioBlue®
V2: 525/50 nm	CD4 VioGreen™	CD4 VioGreen™	CD45 VioGreen™	CD45 VioGreen™
B1: 525/50 nm	CD3 FITC	CD3 FITC	CD3 FITC	CD3 FITC
B2: 585/40 nm	CD16 & CD56 PE		CD19 CAR DR PE	
B3: 655-730 nm	7-AAD	7-AAD	7-AAD	7-AAD
B4: 750 nm LP	CD19 PE-Vio® 770			
R1: 655-730 nm	CD14 APC	CD14 APC		
R2: 750 nm LP	CD8 APC-Vio® 770	CD8 APC-Vio® 770	CD4 APC-Vio® 770	CD4 APC-Vio® 770

Method

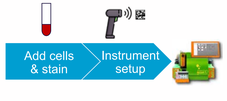
Figure 2

A Comparison of staining workflows
Single liquid reagents:



Dried StainExpress™ tube:

Reduction of manual steps
Risk mitigation
Time saving



B Time saving with StainExpress™

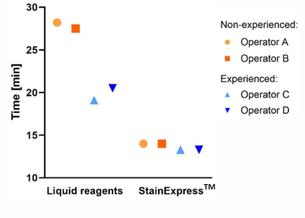


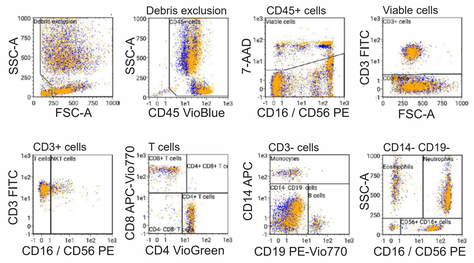
Figure 2a: Experimental workflow used for sample staining before acquisition on MACSQuant 10 or 16. Due to the ready-to-use format of StainExpress™ products, tedious handling steps are eliminated. This mitigates the risk of human error and minimizes day-to-day and operator variability. Figure 2b: By using StainExpress™, the staining procedure is tremendously speeded up, for experienced and non-experienced operators. 6 samples were stained per operator. The time was measured for the whole staining procedure including reagent handling, pipetting, 10 min incubation, and documentation.

Results

1 StainExpress™ provides high lot-to-lot consistency, stability at ambient conditions, and comparability to liquid reagents

Figure 3

A Comparable staining patterns of dried antibody lots: new produced vs. stored for 20 months at room temperature



B Similar frequencies detected with liquid reagents & 3 StainExpress™ lots

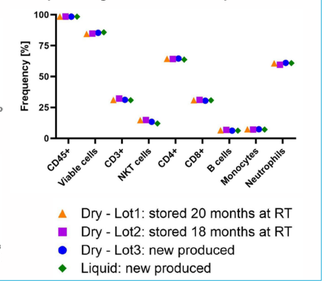
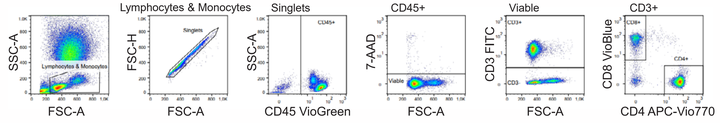


Figure 3: Bulk lysed whole blood was stained with liquid reagents and three different lots of StainExpress™ Immune Cell Composition. Prior to staining, StainExpress™ lots were stored for 20 or 18 months at room temperature (RT) or were newly produced. Figure 3a: Overlay of density plots showing highly comparable staining patterns with StainExpress™ Immune Cell Composition lot 1 and 3. Data analysis was performed with ExpressMode CART_Immune_Composition. Figure 3b: Similar frequencies were detected with all three StainExpress™ lots as well as liquid reagents. Each data point represents the mean of two technical replicates. StainExpress™ products can be stored for 20 months at RT, provide high lot-to-lot consistency and are comparable to liquid reagents.

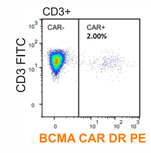
2 StainExpress™ products can be complemented with additional liquid conjugates, CAR Detection Reagents or MHC Multimers

Figure 4

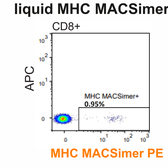
A Representative staining with StainExpress™ T Cell Subsets cocktail



B T Cell Subsets panel with liquid BCMA CAR DR



C T Cell Subsets panel with liquid MHC MACSimer



D T Cell Subsets panel with liquid CD14 & CD19 conjugate

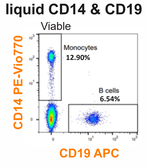


Figure 4: The StainExpress™ T Cell Subsets cocktail offers free fluorescent channels, thus enabling flexible panel complementation: liquid antibody conjugates, CAR detection reagents, or MHC Multimers (MACSimers) were added for a custom-tailored application (marked in orange). Figure 4a: Representative density plots of bulk lysed whole blood (WB) stained with the StainExpress™ T Cell Subsets cocktail. The same gating scheme was used for Figure 4b, c, and d. Figure 4b: BCMA CAR T cells were spiked into bulk lysed WB and stained with StainExpress™ and liquid BCMA CAR detection reagent PE. Figure 4c: Bulk lysed WB from a HLA-A*0201+ Cytomegalovirus-reactive donor was stained with StainExpress™ and liquid MHC MACSimer A*0201 CMV pp65 PE. Figure 4d: Bulk lysed WB was stained with StainExpress™, liquid CD14 PE-Vio770 and liquid CD19 APC.

3 StainExpress™ provides consistent results on different flow cytometers

Figure 5

Comparable frequencies detected with flow cytometers of two different vendors

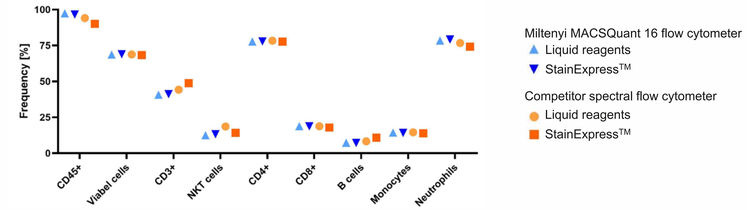
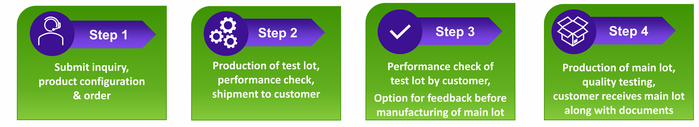


Figure 5: Bulk lysed whole blood was stained with liquid reagents or StainExpress™ Immune Cell Composition cocktail. Samples were acquired with MACSQuant 16 from Miltenyi Biotec and a spectral flow cytometer from another vendor. Similar frequencies were detected with both flow cytometers for samples stained with liquid reagents as well as StainExpress™.

4 StainExpress™ Design: Dried antibody cocktails customized for any application need

Figure 6

A StainExpress™ Design order and production process



B In-process testing ensures high comparability of liquid reagents and dry test & main lot

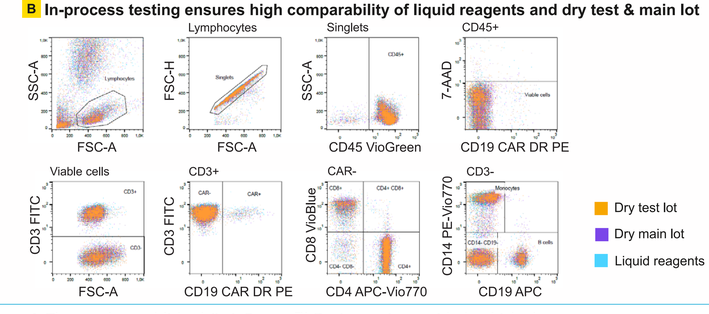


Figure 6: The newly established StainExpress™ Design process allows the production of customized dried antibody cocktails usable for the wide variety of flow cytometric cell analysis applications. Figure 6a: Schematic representation of the steps in the StainExpress™ Design order and production process. Customers are supported by Miltenyi sales representatives with product configuration and the order process. To test the product's performance in advance, a test lot is provided, which the customers can test with their specific application. This gives the opportunity to provide feedback before manufacturing of the main lot. Figure 6b: CD19 CAR T cells were spiked into bulk lysed WB and stained with liquid reagents as well as the customized StainExpress™ Design test lot and main lot. Highly comparable staining results are ensured by in-process testing during manufacturing.

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