

# Counterflow Centrifugation: An Innovative Approach for Efficient Separation and Purification of Cell-Based Products

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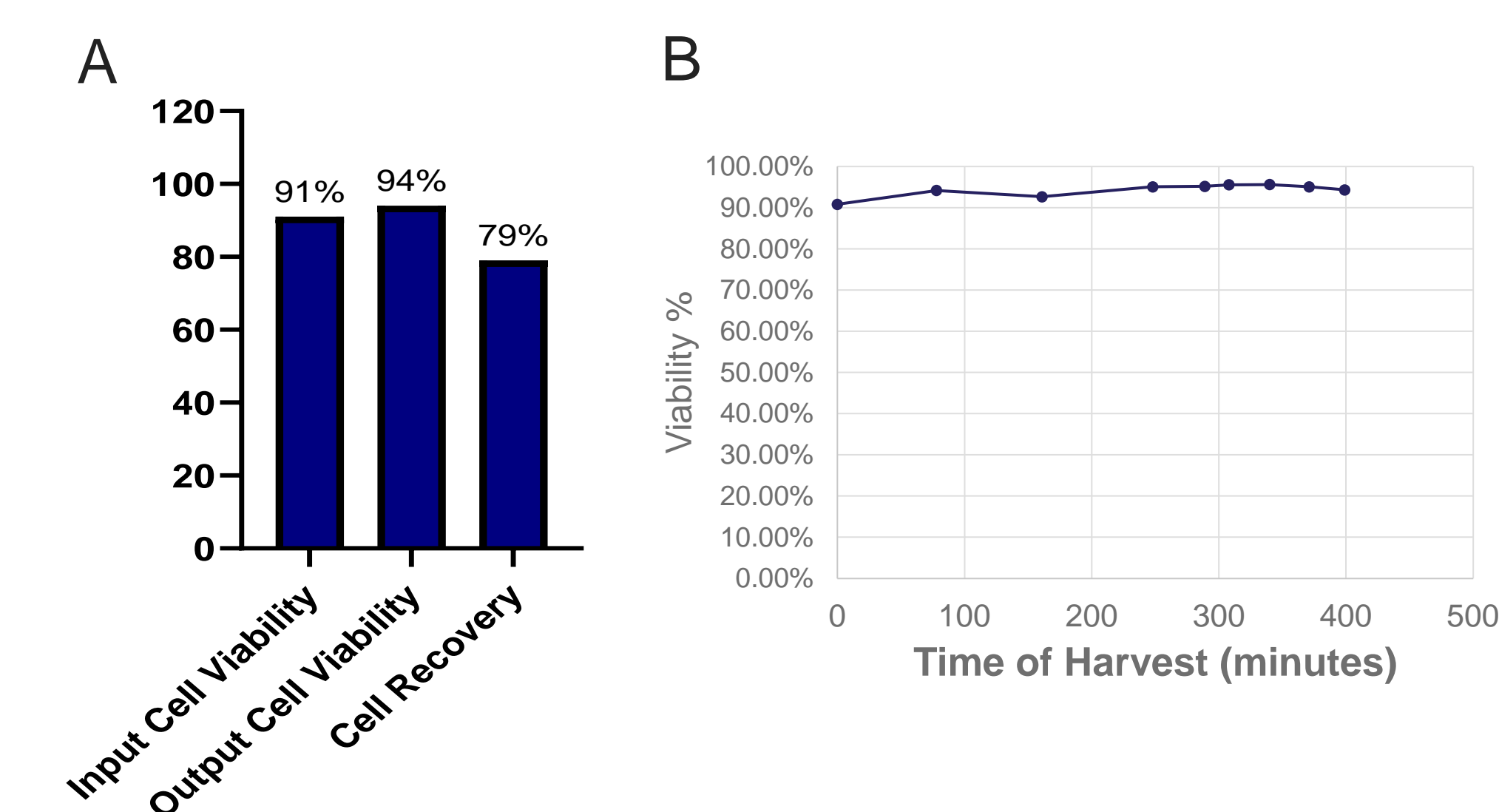
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## Introduction

Gibco™ CTS™ Rotea™ system is a counterflow centrifugation system that offers improved separation efficiency compared to manual centrifugation. By combining centrifugal force with a counterflow of fluid, the Rotea system enables precise separation of different components within a cell suspension based on their density and size. This technology provides increased throughput, reduced processing time, and improved reproducibility. The versatility of the Rotea system is highlighted by its ability to handle large volumes of starting material and higher processing flow rates, making it suitable for industrial-scale production. It is compatible with a wide range of cell types and applications, making it a valuable tool for biopharmaceutical companies, research institutions, and clinical laboratories.

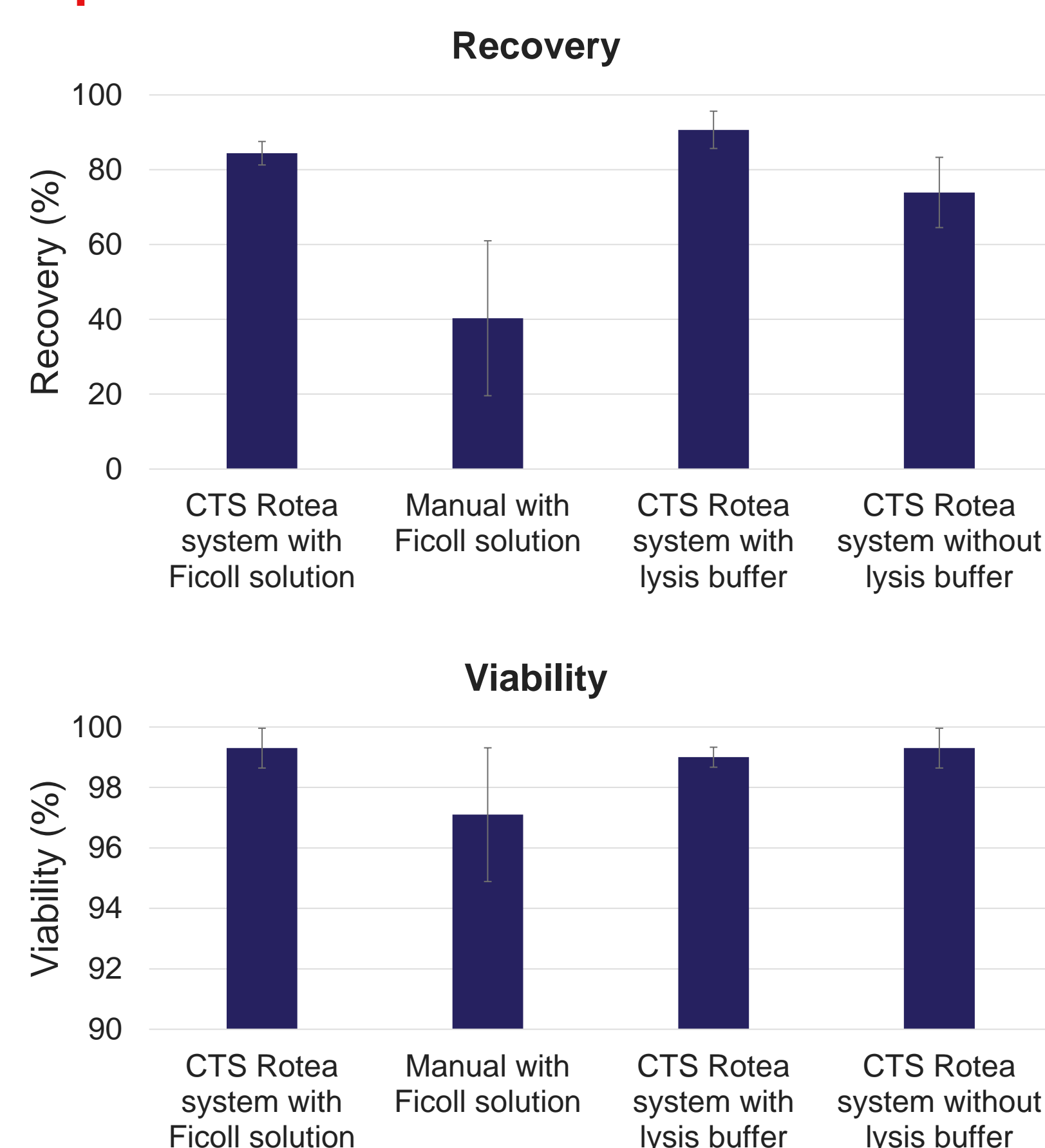
## Application Data

### 1. Large Volume (20L) T cell Wash and Concentrate Run



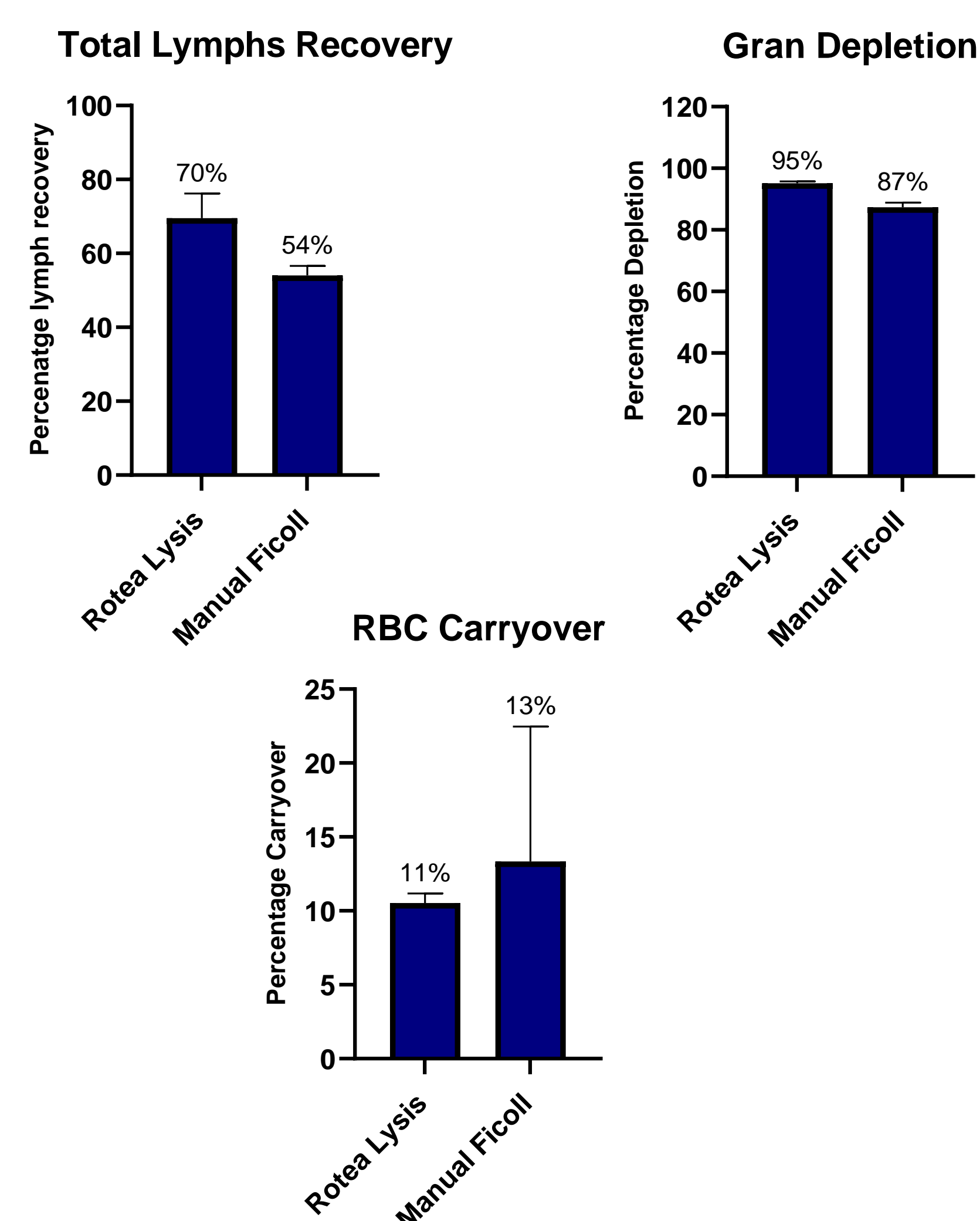
**Figure 1: Recovery and viability of washed and concentrated T cells in a 20L suspension.** A) The viability of the T cells were improved after the processing in Rotea system with excellent cell recovery. B) The 20L cell suspension composed of ~27 billion cells were processed in a “multi-loop” protocol wherein a batch of cells were washed, concentrated and harvested in loops.

### 2. Methods for Isolation of PBMCs from Fresh Leukopak

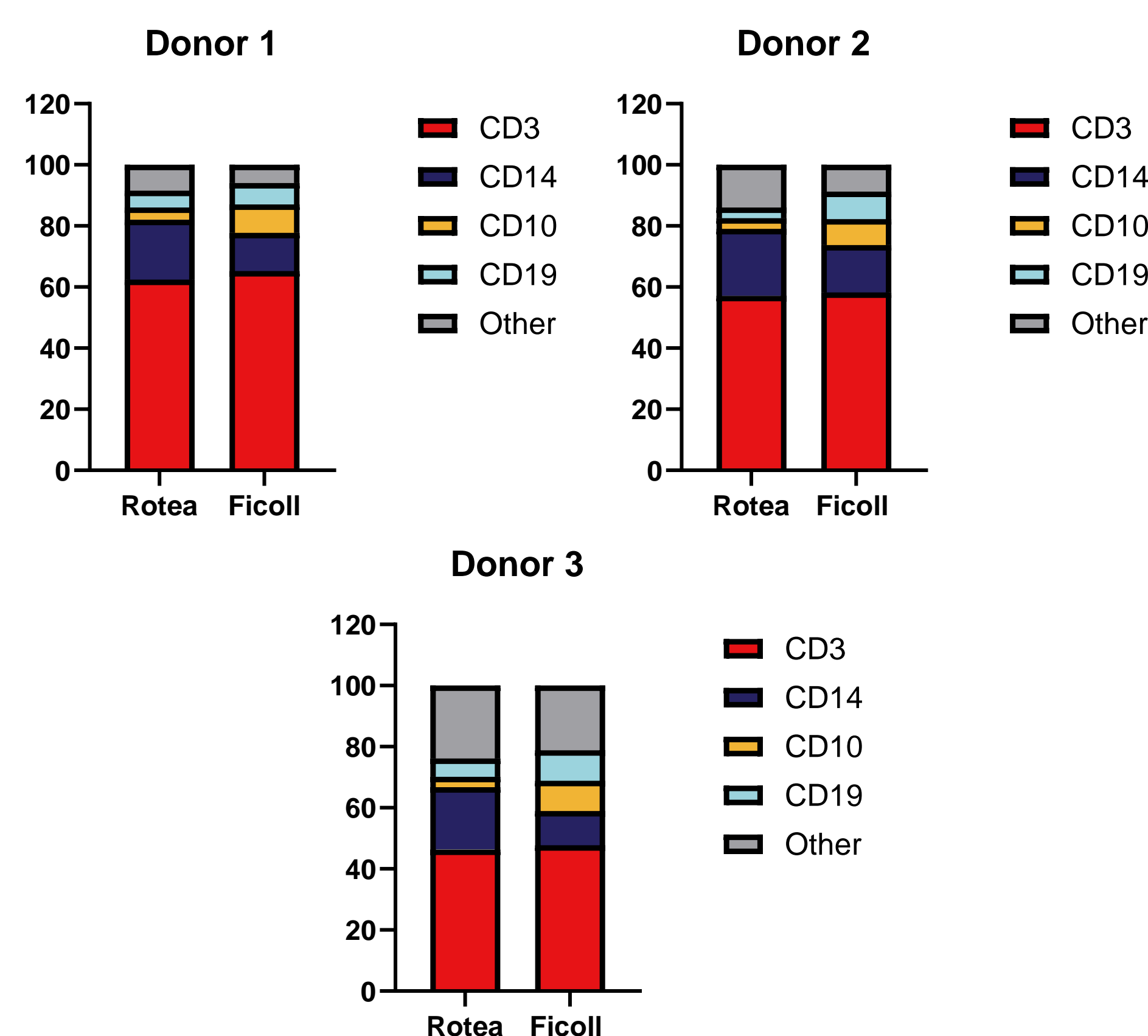


**Figure 2: Recovery and viability of isolated PBMCs from fresh leukopaks using the Rotea system.** ACK Lysis Buffer Method, Ficol Method and No Lysis/ RBC Elutriation method were the 3 different methods used to isolate PBMCs using the Rotea system. These Rotea protocols were compared to isolation of PBMC using a manual Ficol method.

### 3. PBMC Isolation from Whole blood with Granulocyte Depletion



**Figure 3: The Rotea system protocol to isolate the PBMC from whole blood was done using ACK lysis buffer to deplete Red Blood Cells. Granulocytes were separated using the elutriation method wherein the G-force and flow rate of the system was set only to retain the bigger granulocyte in the chamber and elutriate the smaller lymphocyte to a harvest bag.**

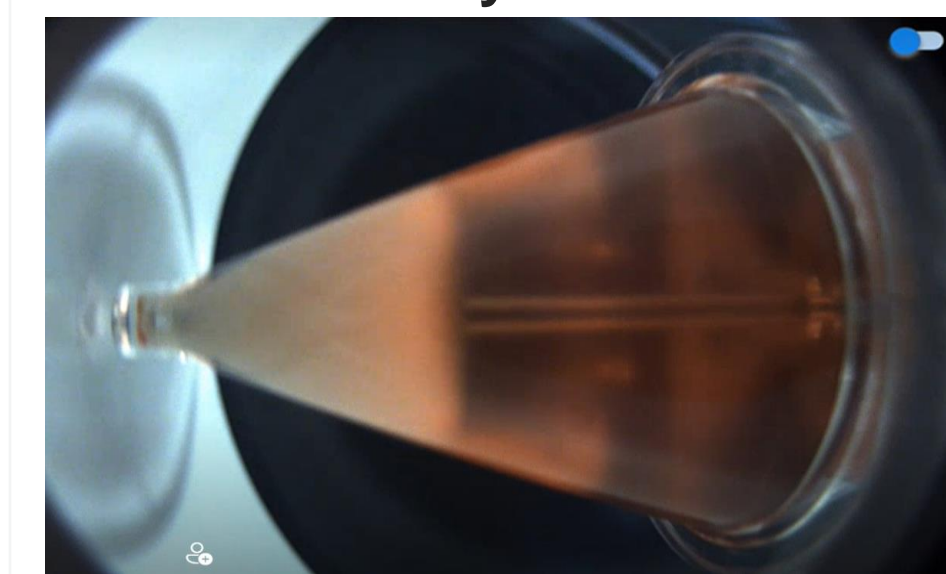


**Figure 4: Phenotype comparison between the Rotea system Lysis protocol and the manual Ficol isolation of PBMC.** For both Rotea and ficoll isolated cells, the CD3 population mostly stayed the same post-isolation.

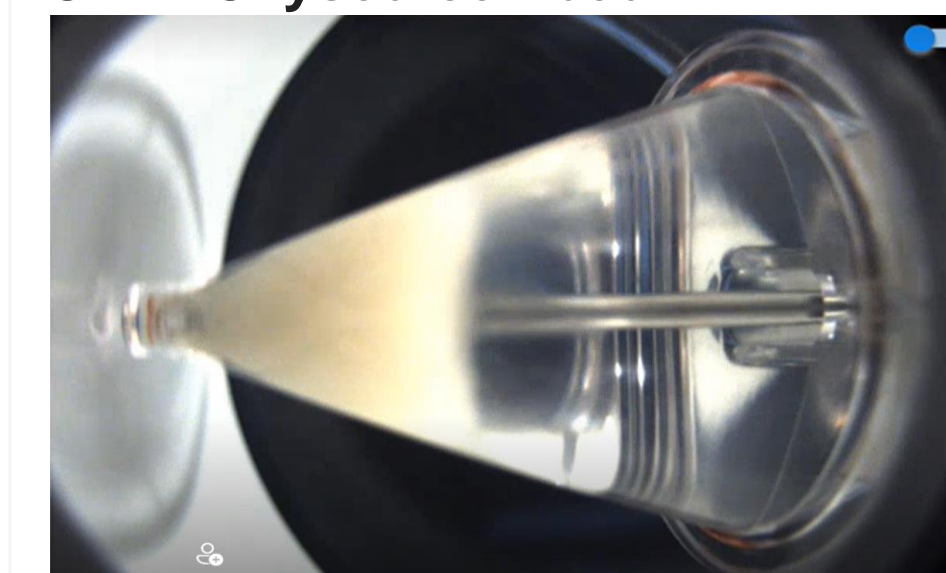
### A: Pre-lysed whole blood



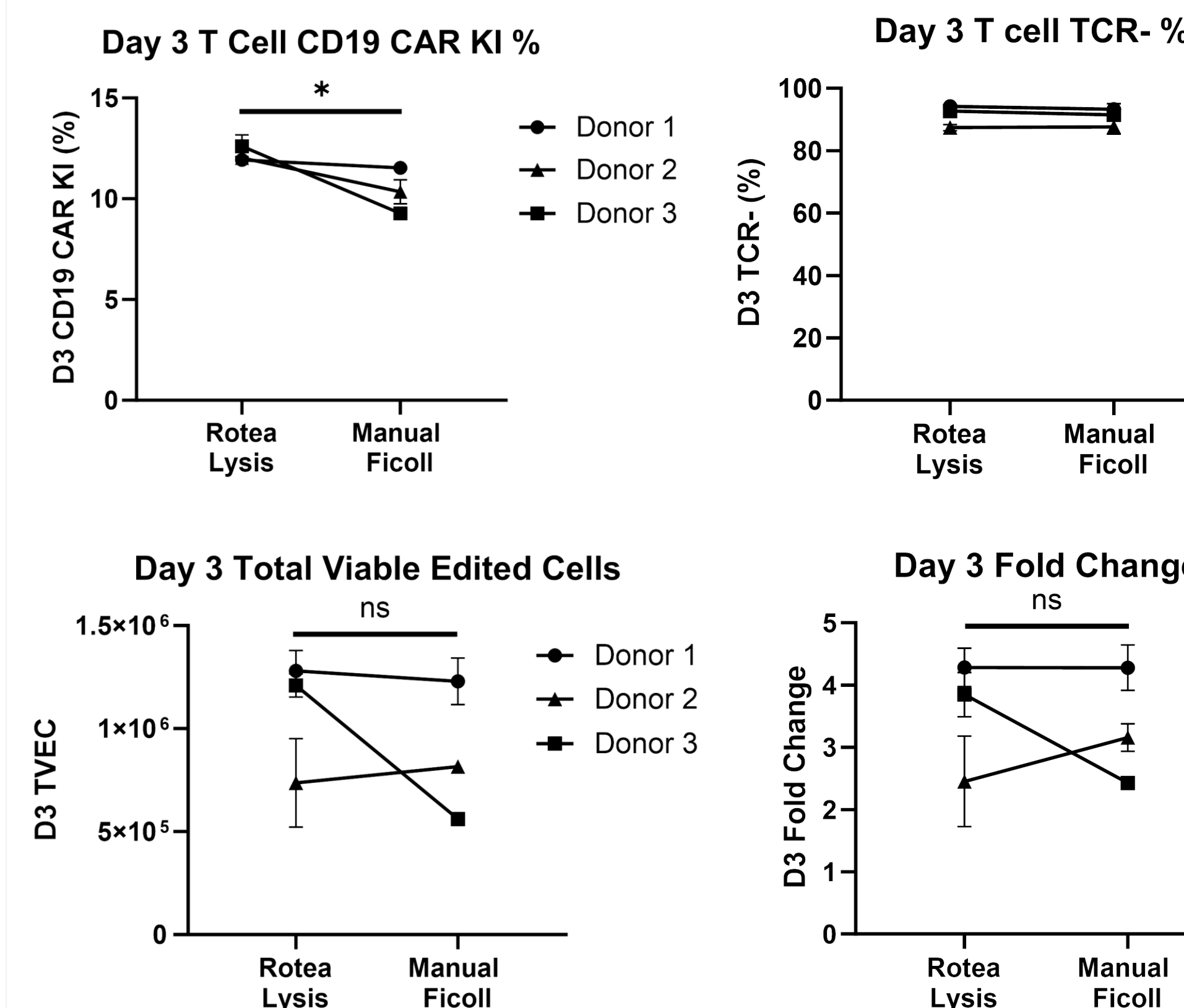
### B: In-chamber lysis



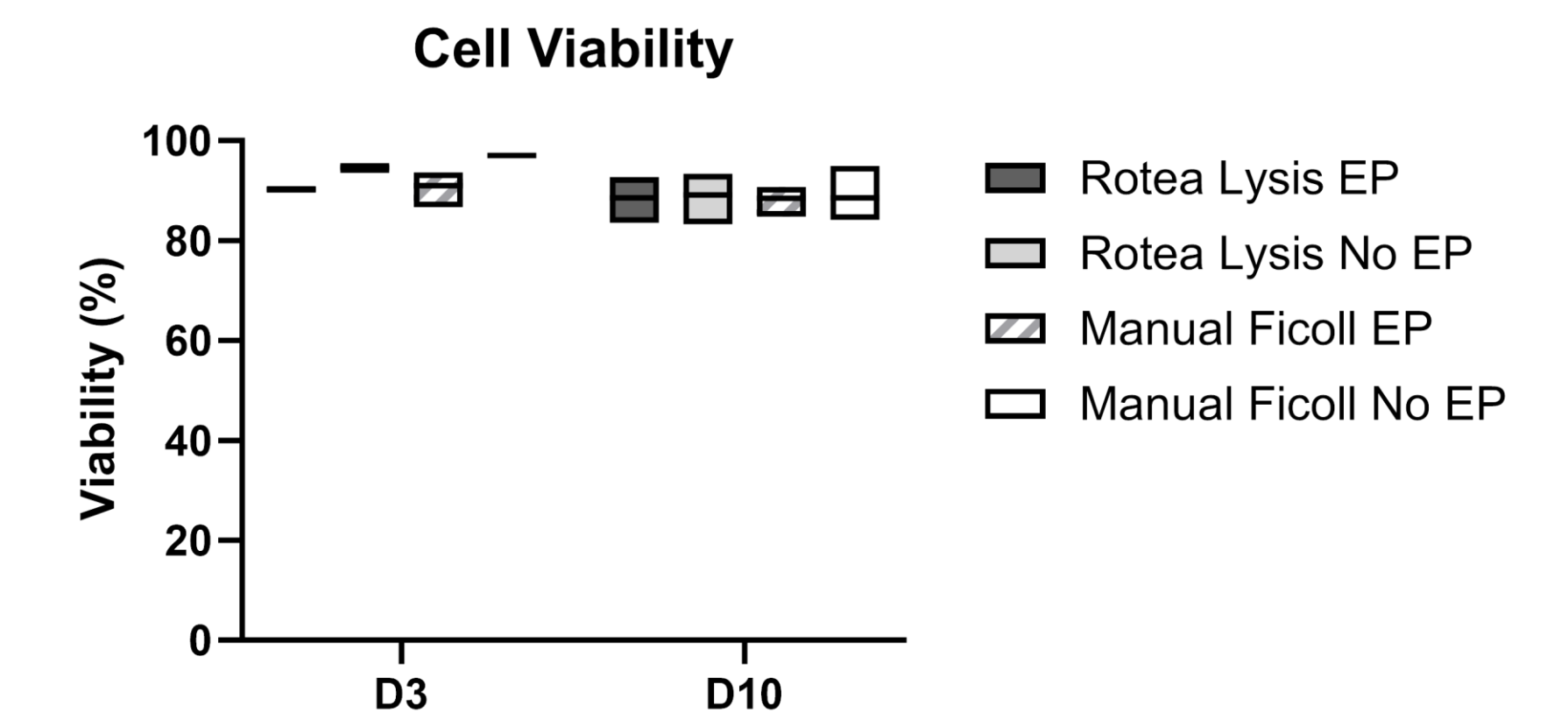
### C: RBC lysed cell bed



**Figure 5: Gibco™ CellCam™ video technology in the CTS Rotea system played a pivotal role in optimizing the protocol development process.** By providing real-time visualization and continuous monitoring, the camera empowers users to optimize their protocols with precision. By closely monitoring the cell bed during the lysis process, users could determine if the chosen volume of ACK lysis buffer was adequate for achieving the desired level of RBC lysis. The camera also allowed for the continuous monitoring of the cell chamber for any potential cell loss throughout the protocol.



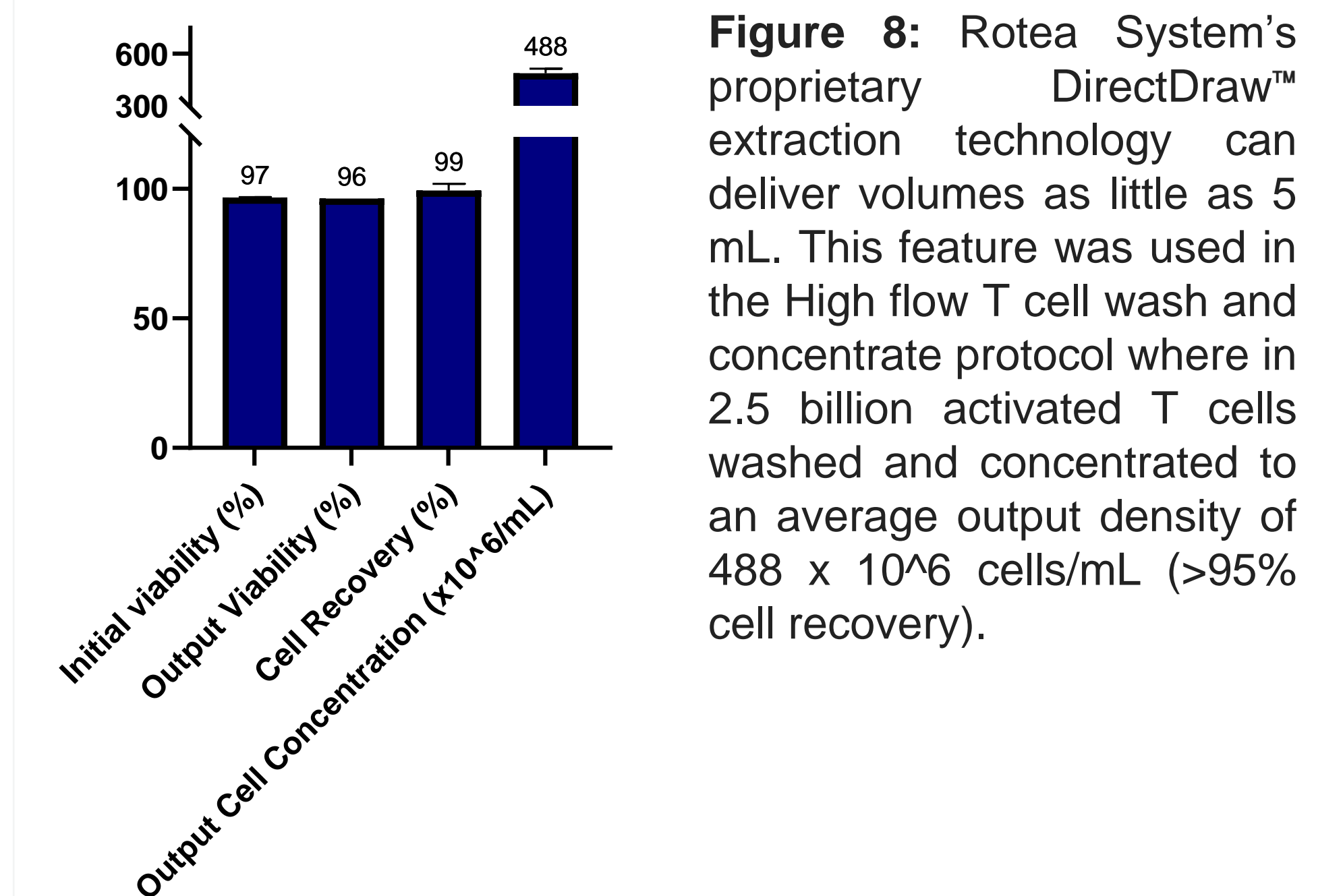
**Figure 6: The isolated PBMCs from both the Rotea lysis protocol and the manual ficoll method were electroporated to measure the efficiency of CRISPR Knockout at TRAC locus and CRISPR Knock-in of CD19 CAR.** PBMCs isolated by Rotea Lysis performed similarly to Manual Ficol in gene editing and expansion metrics.



**Figure 7: Rotea and Ficol EP and No EP conditions maintained high viability and expansion from Day 3 to Day 10.**

### 4. Achieve High Density Cell Harvest using CTS Rotea System

#### Maximum Cell density achieved



**Figure 8: Rotea System's proprietary DirectDraw™ extraction technology can deliver volumes as little as 5 mL.** This feature was used in the High flow T cell wash and concentrate protocol where in 2.5 billion activated T cells washed and concentrated to an average output density of 488 x 10<sup>6</sup> cells/mL (>95% cell recovery).

## Conclusion

The Gibco™ CTS™ Rotea™ system represents a significant advancement in cell therapy manufacturing, addressing the limitations and challenges of manual processes. The Rotea system offers improved separation efficiency, increased throughput, and reduced processing time compared to manual centrifugation methods. The integration of proprietary technologies such as DirectDraw™ extraction and Gibco™ CellCam™ video technology enhances the system's capabilities, enabling efficient cell harvest in minimal volumes and providing real-time visualization for optimization and monitoring purposes. Overall, the Gibco™ CTS™ Rotea™ system offers a comprehensive and reliable solution for biopharmaceutical companies, research institutions, and clinical laboratories seeking efficient and scalable cell processing solutions. By addressing the challenges of contamination, batch to batch variation, higher costs, and limitations in processing volume, the Rotea system contributes to advancing the field of cell therapy manufacturing and supports the industry's move towards allogenic products.

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