From Fresh to Frozen and Everything in Between: Revolutionizing WBC Isolation from Leukopak and Whole Blood with the Rotea Counterflow Centrifugation System

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Introduction

The cell therapy market is expanding rapidly, with numerous companies and academic institutions utilizing a variety of starting materials to produce final cell therapy products. These starting materials, which include fresh or frozen leukopak and whole blood, vary significantly in composition. This variability presents a challenge in identifying a single process or instrumentation capable of efficiently processing all these different materials. As the market continues to grow, the demand for flexible and efficient processing instruments becomes increasingly critical.

The Rotea Counterflow Centrifugation System addresses this need by offering a versatile solution for isolating white blood cells (WBCs) from multiple sources. Whether the starting material is whole blood, fresh leukopak, or frozen leukopak, the Rotea system demonstrates minimal viability loss and exceptional cell recovery. This study showcases the outstanding performance of the Rotea system in processing diverse starting materials, emphasizing its potential to meet the evolving demands of the cell therapy market.

The counterflow centrifugation system was used to isolate the WBCs using the 3 different source material with multiple donors to account for the donor to donor variation. The cell recovery and viability pre and post processing was analyzed using an automated counting instrument and the phenotype pre and post processing was measured using flow cytometry. Cells post isolation were also cultured to make sure of no adverse effect in the expansion process.

For fresh leukopak, the system offers three distinct methods to isolate white blood cells, employing different reagents. These methods cater to customers using legacy reagents such as Ficoll and introduce new approaches with ACK lysis buffer for RBC lysis and WBC isolation, achieving a cell recovery rate of 91%. Phenotypic analysis before and after processing indicates no significant differences.

Fresh Leukopak Processing using the Rotea System

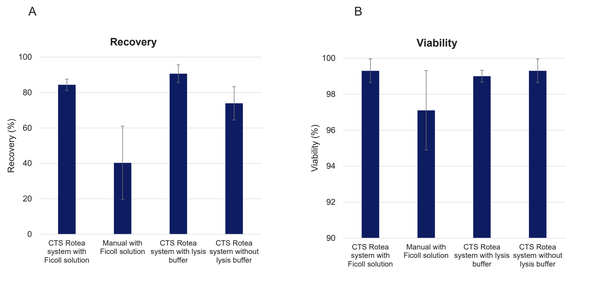

Figure 1: Cell Recovery and cell viability of the isolated PBMCs from the Rotea Counterflow Centrifugation process is shown above. ACK Lysis Buffer Method, Ficoll Method and No Lysis/ RBC Elutriation method were the 3 different methods used along with a control method of manual ficoll to isolate the PBMCs. A) All the three Rotea methods show greater than 75% recovery with ACK lysis buffer method isolation resulting in the highest recovery (91%), while the lowest recovery was observed from the manual ficoll isolation method (40%). The huge variability in the manual ficoll method isolation can also be seen in the graph with the error bar ranging from 20% to 60% recovery. B) Viability of PBMCs immediately following separation was maintained above 97%, with slightly lower viability observed for the manual ficoll isolation.

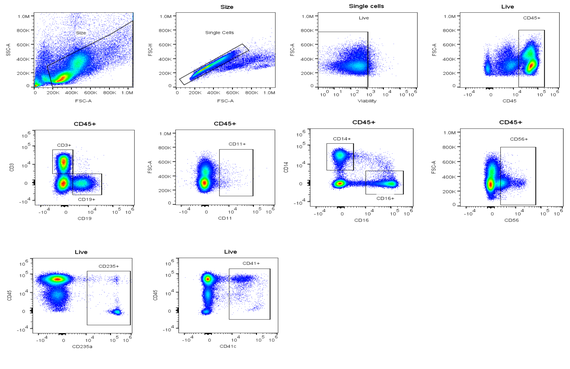

Figure 2. Shows the gating strategy used for the flow cytometry analysis of the PBMCs isolated from the fresh leukopak using all 3 Rotea protocols and the manual ficoll method.

		Frequency (%) of cells				
Cell Type	**Gating Strategy**	**Pre-Processing**	**CTS Rotea System with Ficoll**	**Manual method with Ficoll Solution**	**CTS Rotea System with Lysis Buffer**	**CTS Rotea System RBC Elutriation Method**
Leukocytes	CD45+	79.7	99.6	98.5	99.7	99.8
T cells	CD45+, CD3+	28.5	31.4	25.9	27.1	33.5
B cells	CD45+, CD19+	7.63	9.08	8.69	7.13	8.28
Monocytes	CD45+, CD14+, CD16-	13.4	13.2	16.1	19.6	14.5
Neutrophils	CD45+, CD14+, CD16+	9.15	7.13	8.85	7.53	7
NK cells	CD45+, CD56+	9.77	6.67	8.35	8.76	8.54
Dendritic cells	CD45+, CD11c+	35.9	26.9	36.6	39.4	30.1
Platelets	CD41a+	37.4	21.7	37.4	29.8	26.9
RBCs	CD235a+	10.7	4.68	3.69	2.96	4.74

Table 1. PBMC phenotypes showed after the cells are isolated from Rotea system and manual ficoll method

The table shows the different cell types, the gating strategy and the frequency of the cell types isolated using the 4 different methods. This is also compared against the pre processing sample. As shown, the leukocytes were highly enriched in all the methods. RBC was also depleted considerably in all the 4 methods with ACK lysis buffer methods showing the least amount of RBC Carryover.

Frozen Leukopak Processing using the Rotea System

Frozen leukopaks present more significant challenges in processing compared to fresh leukopaks, primarily due to issues such as cell death caused by freezing, which can result in cell clumping. Additionally, prolonged processing times can exacerbate cell death, as cells remain in cryoprotectant media at room temperature. Despite these challenges, frozen leukopaks are highly preferred in the industry due to their convenience in handling and storage. The Rotea Counterflow Centrifugation System effectively addresses these challenges, offering a robust solution for processing frozen leukopaks. With a processing time of less than an hour for a full leukopak, the Rotea system ensures efficient white blood cell (WBC) isolation. It achieves an impressive 85% cell recovery rate while maintaining cell viability, making it an efficient and reliable option for the industry. This capability underscores the system's versatility and effectiveness in handling the complexities associated with frozen leukopak processing, thereby meeting the industry's demand for efficient and reliable cell processing solutions.

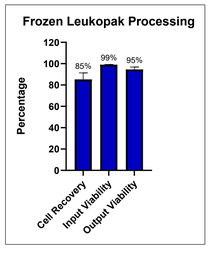

Figure 3. Show the isolation of the PBMCs from a frozen leukopak using the Rotea System. The system demonstrates an exceptional recovery of 85% with minimal change to the cell viability.

Whole Blood Processing using the Rotea System

Processing whole blood can be challenging due to its high red blood cell (RBC) content. However, it is an attractive starting material for those seeking cost-effective alternatives. The Rotea system effectively addresses this challenge with its efficient protocol that utilizes ACK lysis buffer for RBC depletion. After isolating the cells, they are activated using Dynabeads, electroporated with the Neon Nxt system, and then expanded over a period of 10 days.

The study highlights several key data points to demonstrate the efficacy of the Rotea system. These include cell recovery rates, changes in viability, and granulocyte depletion following direct isolation. Additionally, the study provides insights into genetic modification outcomes, reporting knock-in and knock-out percentages, overall viability, fold expansion, and the total number of viable edited cells. These metrics collectively showcase the Rotea system's robust performance in processing whole blood for cell isolation and subsequent genetic engineering.

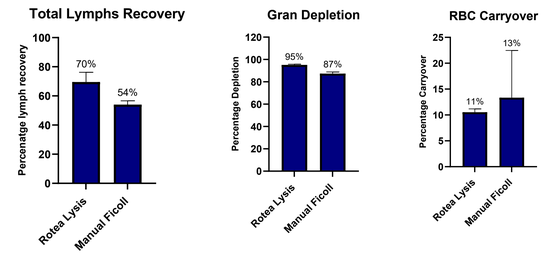

Figure 4: The optimized Rotea protocol was compared with the PBMC isolation using manual ficoll method across 3 different donors. A) The total lymphocyte recovery was much higher with the Rotea protocol compared to the manual ficoll method. B) & C) Rotea lysis protocol also showed good granulocyte depletion and minimal RBC carry over compared to Manual ficoll.
RBC depletion was achieved using ACK lysis buffer and granulocyte depletion was achieved using the elutriation process that is unique to counterflow centrifugation.

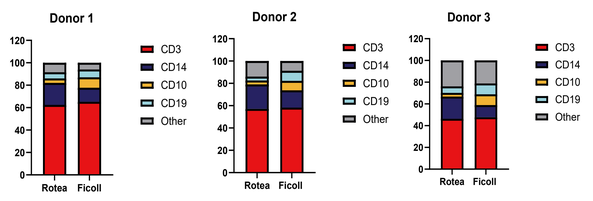

Figure 5: Phenotype comparison between the Rotea system Lysis protocol and the manual ficoll isolation of PBMC. For both Rotea and ficoll isolated cells, the CD3 population mostly stayed the same Post isolation. The outputs from the Ficoll isolated methods have relatively higher granulocyte (CD10+) population compared to the Rotea isolated cells

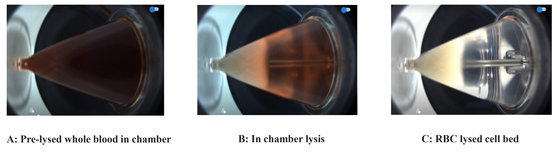

Figure 6: Gibco™ CellCam™ video technology in the CTS Rotea system played a pivotal role in optimizing the protocol development process. By providing real-time visualization and continuous monitoring, the camera empowers users to optimize their protocols with precision. By closely monitoring the cell 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.

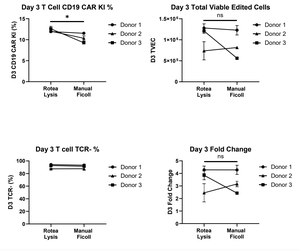

Figure 7: The isolated PBMCs from both the Rotea lysis protocol and the manual ficoll method were electroporated to measure the CRISPR Knock out at TRAC locus and CRISPR Knock In of CD19 CAR. PBMCs isolated by Rotea Lysis performed similarly to Manual Ficoll in gene editing and expansion metrics.

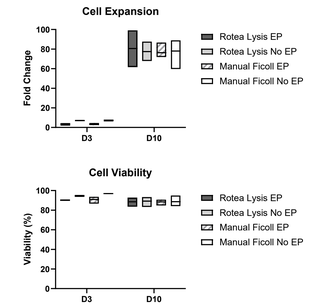

Figure 8: Rotea and manual Ficoll EP and No EP conditions maintained high viability and expansion from Day 3 to Day 10. Although a slight viability drop was noticed with EP samples at day 3 the cells recovered well and EP samples at day 10 showed similar viability and fold change in expansion

Conclusions

The Rotea Counterflow Centrifugation System excels in isolating white blood cells from diverse starting materials, including fresh and frozen leukopak, as well as whole blood. Each material presents unique challenges due to their distinct compositions.
Rotea System offers three different methods to isolate PBMCs from fresh leukopak, with the highest cell recovery rate achieved with the ACK lysis buffer protocol at 91%. It has also been shown that the use of ACK lysis buffer did not have any adverse effect on the cell health, expansion, and its phenotype. For users, not interested in lysis buffer method, Rotea system also offers protocol using Ficoll reagent or no reagent at all.
Frozen leukopak presents challenges like cell death and clumping due to freezing. Despite these hurdles, the Rotea system processes frozen leukopak efficiently, achieving an 85% recovery rate and maintaining cell viability.
Whole blood, with its high red blood cell (RBC) content, demands effective RBC depletion for successful processing. The Rotea system meets this challenge using ACK lysis buffer The isolated cells underwent cell activation, electroporation, and expansion. The system demonstrates strong performance in cell recovery, viability, and genetic modification outcomes.
The Rotea system's versatility in handling different starting materials makes it a valuable tool for the cell therapy market. Its ability to maintain high cell recovery and viability across various compositions positions it as an essential asset for any cell therapy workflow. As the cell therapy industry grows, the Rotea system's flexibility and efficiency will continue to meet the evolving demands of cell processing.

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