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Building New Cell Therapy Workflow Solutions by Leveraging the Flexibility of the Rotea System's Closed Automated Cell Processing Technology

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INTRODUCTION

Processing and manufacturing cell and gene therapy products is of vital importance to patients and has presented numerous challenges. The Gibco™ CTS™ Rotea™ Counterflow Centrifugation System's closed technology enables gentle cell isolation, and wash and concentrate, and can be easily automated and programmed to serve many additional workflows. Together the single use kit and instrument are able to capture and sustain billions of cells in a stable, fluidized bed with flexible inputs up to 20 liters and output volumes as low as 5 mL.

Two of the Rotea system's core capabilities are PBMC isolation from fresh leukopaks, and wash and concentration of T cells, two common and vital processes in cell therapy workflows. Recently, we have utilized the flexible Rotea system to isolate PBMC from frozen leukopaks, and wash and concentrate frzoen PBMC and cultured NK cells.

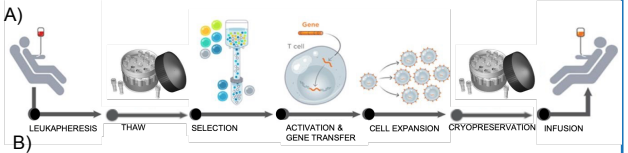

Figure 1. Current cell therapy workflow. (A) Current processes rely heavily on cell isolation, wash, and concentrate. Automated processing in each step indicated in (B) can increase the efficiency and lower the cost of cell therapy manufacturing.

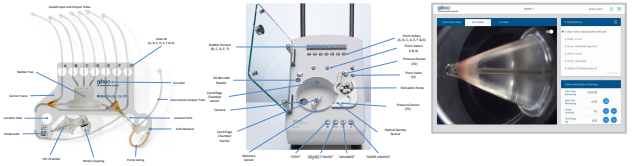

Figure 2. The Rotea system which includes the single use kit, instrument and accompanying software, provides closed and automated cell processing solutions by collecting cells of interest in a fluidized cell bed.

Key Features:

- **Scalable throughput**- continuous processing of up to 20L
- **Small minimum output volume**- As low as 5 mL
- **Flexible**- programmable, multiple processing modes including cell separation, concentration and washing. The system can easily be adapted by the user to new workflows.
- **Universal kit** (consumable) for different cell types and applications
- **Small bench top footprint**

RESULTS

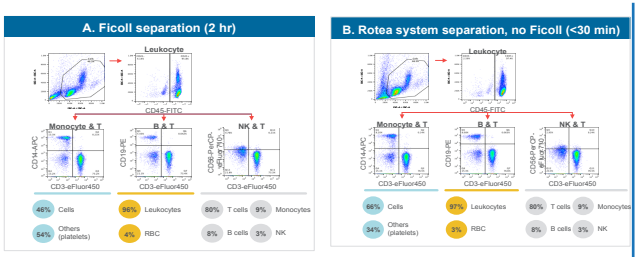

Figure 3. T cells processed using the Rotea system exhibit similar quality as compared to Ficoll control throughout expansion. (A, B) Rotea completes PBMC isolation in 30 minutes with thorough platelet and RBC depletion. Additionally, the composition of cells after processing with the Rotea system is comparable to manual density gradient processing.

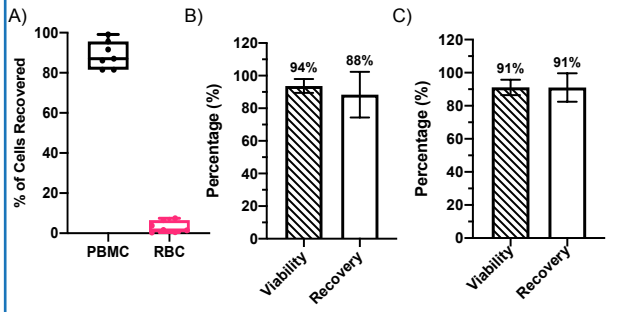

Figure 4. A) PBMC Isolations from fresh leukopak donors using ACK Buffer for RBC lysis. The average PBMC recovery across 7 donors was ~90%. After Isolation and RBC lysis, on average less than 5% of the starting population of RBCs remained. B) **PBMC isolations from frozen leukopak donors**. Average recovery across 2 donors was 88% and viability averaged 94% after processing. **C) Frozen PBMC using the Rotea system to remove cryopreservation medium and wash into growth medium (buffer exchange).** For n=3 Rotea system runs, both viability and recovery of frozen PBMC exceeded 90%.

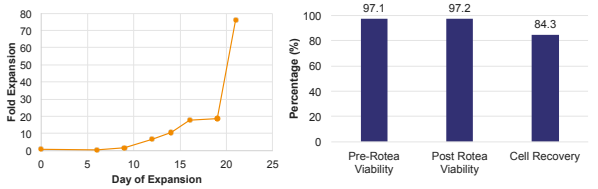

Figure 5. NK cell expansion and subsequent Wash and concentrate performed on Day 21 of expansion. NK cell viability was maintained and cell recovery was over 84% prior to Rotea system protocol optimization.

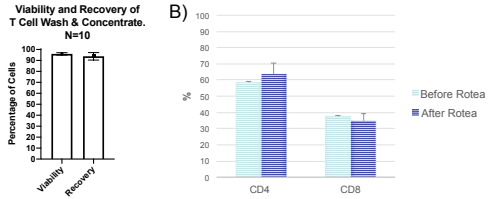

Figure 6. Rotea system T cell wash and concentrate with controllable input and output. A) T cell recovery and viability over 10 runs of T Cell wash and concentrate, both recovery and viability averaged over 90% with low variability. **B) Phenotype of T Cells both before and after T cell wash and concentration with the Rotea system.** The composition of CD4+ and CD8+ cells is not altered after Rotea system wash and concentrate.

CONCLUSIONS

- The Rotea system provides highly efficient PBMC isolation from both fresh and frozen leukopaks across many donors with high recovery and cell quality. It can isolate PBMCs much faster than manual methods while maintaining equivalent or improved quality and cell health
- The Rotea system provides gentle and efficient cultured T cell, and frozen PBMC wash and concentrate with >90% recovery of T cells, and maintenance of viability with flexible input and output volume. The system is also capable of performing wash and concentrate of NK cells with high viability and recovery
- Due to it's high level of flexibility and programmability, the Rotea system is capable of facilitating many important aspects of cell therapy workflows, from PBMC isolation all the way to cryopreservation

TRADEMARKS/LICENSING