

Streamlined Cell Therapy Manufacturing: Highlighting Workflow Flexibility and Functionality with Advanced Processing and Fill Finish Instrumentation

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Introduction

The advancement of cell therapy manufacturing necessitates robust, flexible, and efficient systems to ensure high-quality outcomes for patients. This study the concentration, formulation, and fill and finish steps of the cell therapy workflow using semi-automated instrumentation. For cell processing including wash and concentrate steps, the Gibco CTS Rotea Counterflow Centrifugation System was used while for Fill and Finish the Gibco CTS Compleo Fill and Finish System was used.

We set out to perform an investigative study of the two instrument specifications to evaluate and illustrate their capabilities for use in cell therapy manufacturing workflows. To do this we evaluated the CTS Rotea systems ability to wash and concentrate primary T cells at starting volumes of <1 Liter and of 20 Liters (system maximum). We also tested the instrument's ability to provide low volume outputs (down to 5mL) at high cell concentrations (up to 5e8 primary T cells/mL). The ability to perform low volume, high cell concentration harvests is unique and particularly beneficial for applications preceding electroporation or cryopreservation. For the fill finish steps, we tested the CTS Compleo System's ability to deliver accurate outputs at both low (2mL) and high (750mL) volumes. We also compared the accuracy using 2mL output volumes across instruments and outputs lines. Finally, we checked cell concentration across 3 instruments. Both the CTS Rotea System and the CTS Compleo System were analyzed independently to understand their capabilities and potential applications in the field as part of the larger workflow.

The evaluation was conducted through a series of workflow-based experiments designed to assess the core functionalities of the two instruments.

Results

CTS Rotea Counterflow Centrifugation System: Primary T Cell Wash & Concentrate



Cell Processing using Counterflow Centrifugation

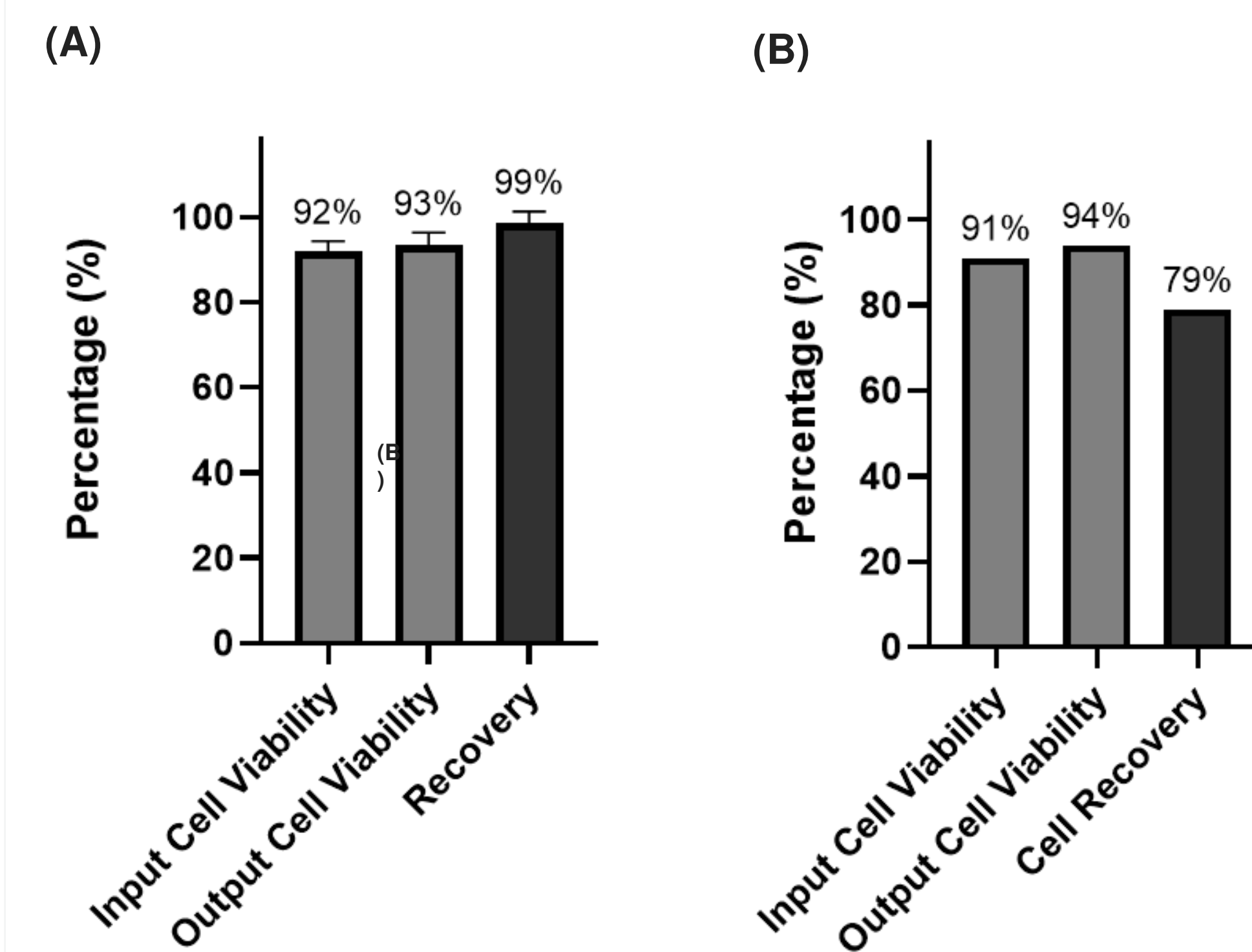
Process flexibility—optimization of protocols for cell separation, wash, and concentration

Low output volumes—as little as 5 mL

High cell recovery and viability—enabling >95% cell recovery with no loss in cell viability

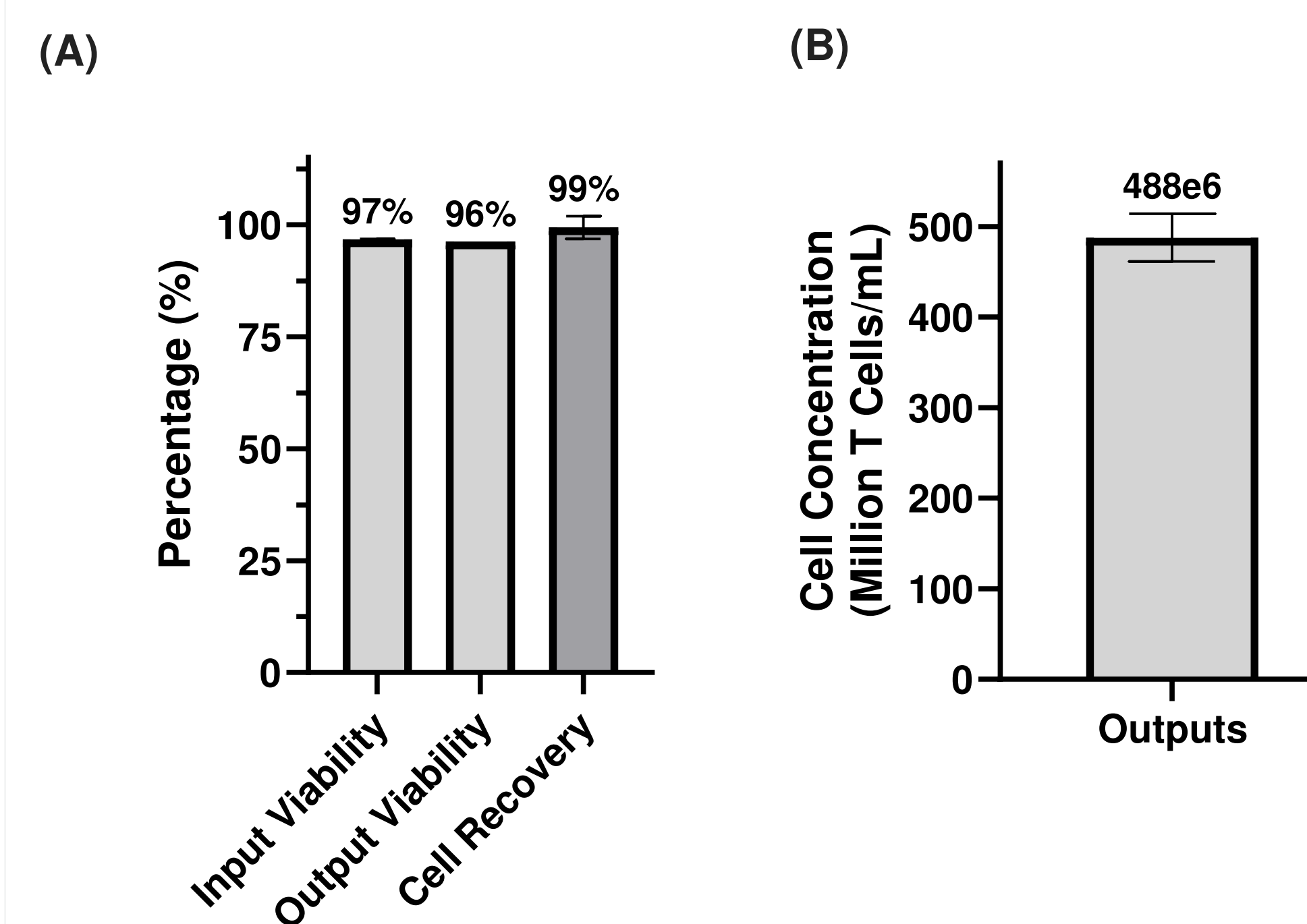
When processing large volume cell suspensions, it is essential to maintain or improve cell viability post-processing. Additionally, a smooth transition from process development to commercial manufacturing is crucial. The CTS Rotea system addresses these challenges with a cell processing range of up to 20 liters (Figure 1) and the capability to handle 1-5 billion T cells within the single use consumable cone. The fluid path and protocol design allow for performing multiple loops and therefore processing much more total cells than can fit into the cone within a single protocol. Additionally, the system can provide outputs as low as 5mL, with very high cell concentrations up to 5.06e8 T cells/mL. (Figure 2). These features are key to transition cells into subsequent downstream steps such as electroporation or cryopreservation and provide general flexibility to suit whatever process is required.

Figure 1. CTS Rotea System results for processing (A) <1 liter and (B) 20 liters of primary T cells using the same wash and concentrate protocol.



For both volume ranges, viability was equivalent or increased compared to pre-processing. At low volumes below 1 liter, cell recovery averaged 99% for 7 runs. At 20L, cell recovery was 79% for a single run. In order to provide an exact scaling comparison, identical protocols were used for both the <1 liter and 20 liter runs, however, with minor optimization the cell recovery could likely be increased significantly for the 20-liter run.

Figure 2. Viability, cell recovery (A) and cell concentration for 5mL T cell outputs (B) on the CTS Rotea System



Two wash and concentrate runs were conducted with 2.5 billion primary T cells, targeting an output concentration of 500 million cells/mL. The achieved concentrations were 506 million cells/mL and 469 million cells/mL, with an average concentration of 488 million cells/mL.

Note: Both dead volume and pressure in the output line must be accounted for in the harvest volume entered in the software and can be calculated based on tubing length.

Example: For an actual Harvest volume of 5 mL, with an additional 0.7 mL for the dead volume, the entered Harvest volume should be 6.2 mL (5 mL + 0.7 mL + 0.5 mL).

CTS Compleo Fill and Finish System: Volume accuracy, precision and instrument consistency



Closed, automated system for final formulation and filling

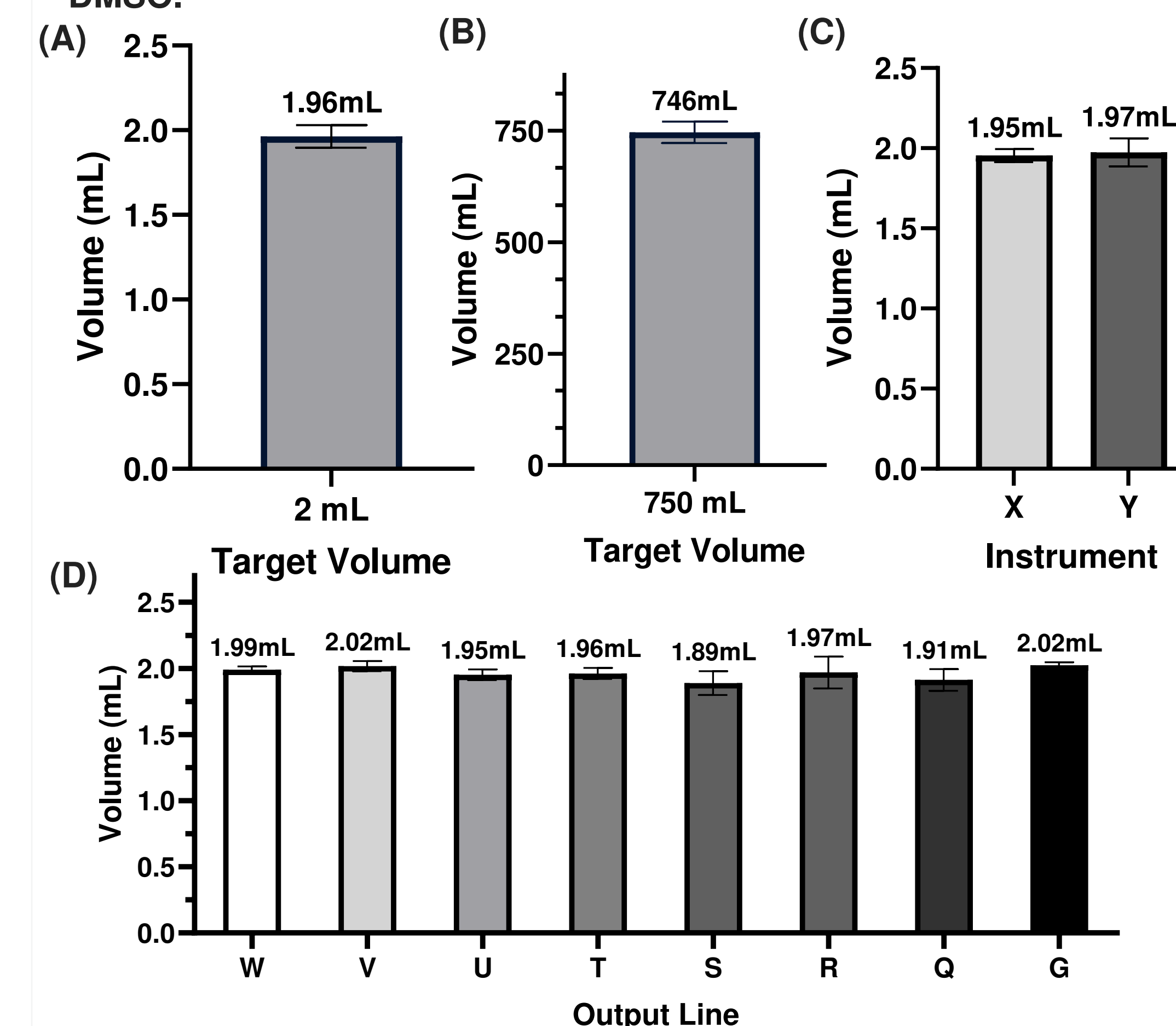
Broad Flexibility—Up to 4 inputs and 10 outputs.

Consistent Accuracy — Support output volumes between 2mL and 750mL.

Process Compatibility—Compatibility with bags and vials.

As stated in the introduction section, the CTS Compleo Fill and Finish system provides a closed, automated system for final formulation and filling. As with the CTS Rotea system, the CTS Compleo System is focused on flexibility with up to 4 inputs at volumes of up to 750mL total volume and 10 outputs of 2mL to 750mL. The system also allows for compatibility with both bags and vials to be used as output containers and comes with an attached mixing bag to ensure effective mixing and homogeneity using the paddle mixer component. Additionally, the system can remove air from output containers and perform calculations to ensure dose parameters are achieved for the specified process.

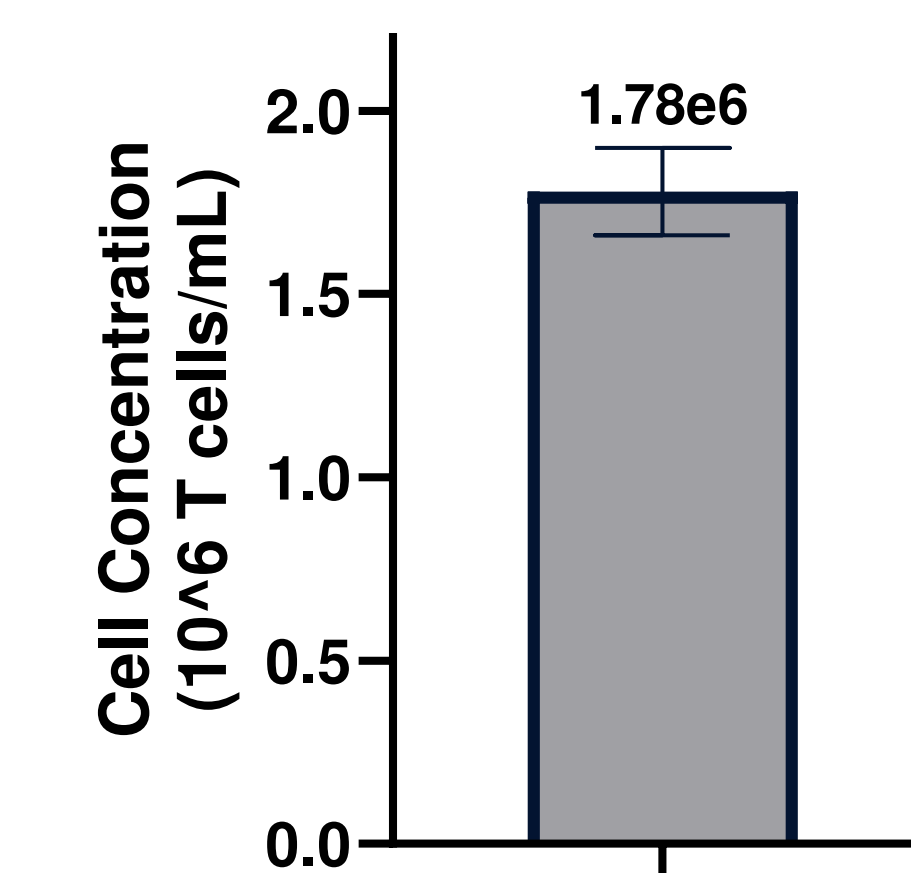
Figure 3. Volume accuracy for 2mL outputs (A), 750mL outputs (B), two instrument accuracy comparison for the 2mL volume output (C), and accuracy comparison across 8 different outlines lines on the same instrument for the 2mL output volume (D). All runs shown were performed with primary T cells in Cryopreservation Media with 10% DMSO.



On two different CTS Compleo Systems, cells suspended in cryopreservation buffer with 10% DMSO and output were delivered at the specified target volumes. For target volumes of 2mL (A), the average output volume was 1.96mL with an average percent error of 4.4%. For 750mL (B) the average output volume was 746mL with an average percent error of 5.2%. These results highlight the flexible nature of the CTS Compleo System in terms of volume accuracy at both small and larger output volumes.

We then separated the 2mL output data by instrument and looked at the variation between the two instruments (C). The difference in average output volume between the two instruments was 0.02mL. Finally, we looked at each output line on a single instrument in triplicate and compared the results. The average volumes varied from 0.01mL to 0.11mL from the target of 2mL. Together this data highlights the volume accuracy of the instrument across instruments, output lines, and the output possible volume range. As this instrument is in development, further work will be done to characterize the low volume range including output volumes below 2mL.

Figure 4. Cell Concentration for 750mL output volumes using primary T cells in Cryopreservation Media with 10% DMSO



For the runs shown in Figure 3b, the cell concentration was also recorded and compared against the target cell concentration. A total of 10 outputs were used for this purpose across 2 different instruments. The final cell concentration of the 750mL output was compared directly against the expected cell concentration which was determined for each output based on the input cell concentration. The average target cell concentration was 1.71e6 T cells/mL while the average actual cell concentration was 1.78e6 T cells/mL, yielding an average percent error across all 10 outputs of 4.5%. The cell concentration data has the added error associated with cell counting which adds an additional layer of potential variability unrelated to the accuracy of the instrument itself. This ability to deliver not only accurate volumes but also consistent cell concentrations, shows that the CTS Compleo system has an effective mixing system as well as high cell yield from the process.

Conclusion

Both the CTS Rotea System and the CTS Compleo System serve to benefit cell therapy manufacturing processes through their closed, automated and flexible capabilities. Here we show that both instruments can work with varied input and output volumes. We showed the capability of the CTS Rotea System to process <1 liter and 20 liters of primary T cells while maintaining cell viability above 96% and maintaining cell recovery 79%+ without any protocol adjustments between the two volumes. Within the fill-finish steps, high accuracy and recovery. Output samples from 2mL up to 750mL showed results very close to the target volumes and percent errors well under 10% for all tested conditions. These conditions included comparing two different instruments, 8 different output lines across a single instrument, and low and high volume outputs of 2mL and 750mL respectively. Lastly, cell concentration for 2mL outputs was shown to have similar accuracy to volume alone, highlighting the effectiveness of the paddle mixing system and robustness of the instrument protocols. Both instruments exhibit significant strengths in their respective functionalities, offering valuable tools for cell therapy manufacturing. This evaluation highlights their potential to enhance product yield and consistency in manufacturing. The flexibility in input and output volumes for both instruments and the accuracy and precision during fill-finish operations, underscore the instrument's effectiveness in supporting cell therapy manufacturing. Additional work is in progress to explore further workflow integration of these systems. For more information, please visit thermofisher.com/compleo.