# Low Volume Wash, Formulation and Fill Strategies for T Cell Therapies

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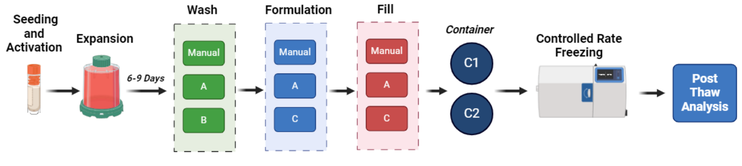
## Abstract

While many approved cell therapies typically infuse between 50 - 70 mL or more, smaller dose aliquots are increasingly being used. Efforts in the cell therapy space, directed towards improving cell potency and persistence, will likely drive further reduction in the number of cells administered per dose. Given that there is a minimum cell concentration at which these cells can be cryopreserved while maintaining sufficient post-thaw stability, this requires working with much lower volumes in manufacturing. The accuracy and precision required to wash, formulate, and fill small volumes in a closed system requires new and innovative solutions. Final product containers must also be evaluated for suitability with desired administration procedures and their compatibility with fill equipment. Although instruments capable of processing lower volumes are being developed, there is still limited data characterizing their effective operating ranges and practical limitations.

In this study, we evaluate and optimize state-of-the-art modular solutions for low volume wash, formulation and fill, that can easily be incorporated into existing cell manufacturing platforms. We compare individual instruments for wash, formulation and fill, in tandem with instruments capable of performing two or more operations simultaneously. Critical metrics evaluated here include cell recovery, viability, fill accuracy and consistency, as well as the homogeneity of the formulated cell product. We also assess post-thaw viability of the cell therapy drug product that correlates with stress encountered by the cells during processing. To support the choice of a final product container, we measure the recoverable volume via syringe and needle-less adaptors. We also assess the ease of clinical handling and administration. Finally, we provide a cost and time assessment to help drive a complete understanding and identify gaps for future development.

## Method and Materials




T cells were activated and expanded for 6-9 days. A total of 3 runs were performed on each wash device using cells from a single donor, followed by different formulation and fill methods. For fill, a minimum of 4 replicate containers were analyzed. Instrument A was assessed for wash, formulation and fill performance. Instrument B was tested for wash and instrument C for formulation and fill performance. cells. Post fill, the cells were frozen in a controlled rate freezer and stored in LN2 until post-thaw analysis.

- **Cell input:** 150 – 200 X $10e^6$ Total Viable cells
- **Target wash output:** 10 mL
- **Formulation** 1:1 with CS10
- **Target formulated output**: 20 mL
- **Target fill volume**: 4 mL

*Graphs and statistical analysis performed on Graphpad Prism. Illustrations created using Biorender.*

## Results

### Wash option A showed advantages in terms of cell recovery and post-thaw viability.

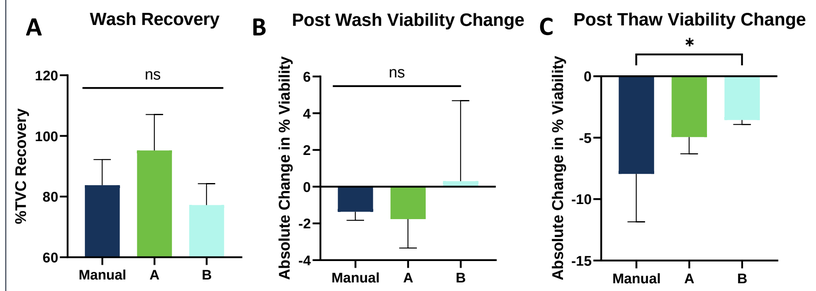



**Figure 1: Wash performance of Platform A,B and Manual Wash.** a. Post wash TVC recovery b. Change in viability post wash compared to input. N = 3. C. Change in viability post-thaw compared to post-wash. N = 4-5 Data analyzed using one-way ANOVA, n.s. not significant, *P<0.05

### Automated fill through C led to superior fill accuracy and homogeneity.

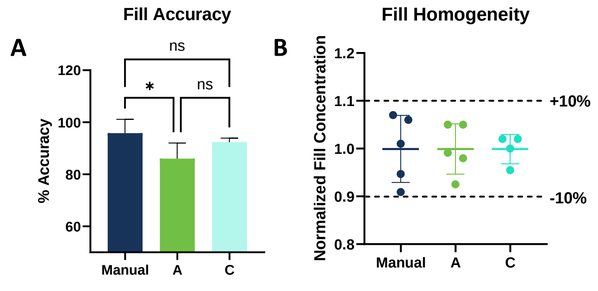


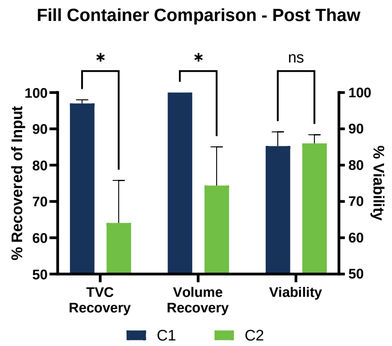

**Figure 2:** Fill performance of Platform A, C and Manual Fill. A. Fill accuracy of different methods. B. Concentration across different fill containers normalized to average concentration. Dotted line indicates ±10% variability Data analyzed using one way ANOVA, N = 4-9. n.s. not significant, *P<0.05

### Cost and Time Assessment

| Wash | Formulation | Fill | Recovery | Cell Stability | Fill Accuracy | Capital Cost | Time |
|---|---|---|---|---|---|---|---|
| Manual | Manual | Manual | ● | ● | ● | ● | ● |
| A | A | A | ● | ● | ● | ● | ● |
| B | B | Manual | ● | ● | ● | ● | ● |
| A | A | Manual | ● | ● | ● | ● | ● |
| A | A | C | ● | ● | ● | ● | ● |

● Favorable ● Unfavorable

### Container C1 is superior in terms of recovery and cell stability for low volume fill




| Container | Handling | Dead volume | Integrity | Cost |
|---|---|---|---|---|
| C1 | ● | ● | ● | ● |
| C2 | ● | ● | ● | ● |

● Favorable ● Unfavorable

**Figure 3: Container comparison.** TVC Recovery, Volume recovery and viability post thaw for containers C1 and C2. Data analyzed using Mann-Whitney test. N = 5. n.s. not significant, *P<0.05

## Discussion

Three instruments were evaluated separately for wash, formulation and fill of a low starting cell number into a low output fill volume. Cell recovery post wash was highest for instrument A, although this difference was not statistically significant. Both automated wash processes led to higher cell viability post-thaw, indicating lesser stress on the cells compared to manual wash. Overall data indicated platform A to be the better automated option for wash and formulation, compared to B. Fill volume accuracy of instrument C was comparable to manual fill, whereas A had significantly low accuracy compared to manual fill. The fill homogeneity between container replicates was highest for C, further supporting its choice as an automated fill device. Two fill containers were assessed for low volume fill. In terms of cell stability and overall recovery, container C1 was evidently superior to container C2. Further, C1 was better than C2 in terms of ease of handling and container integrity.

Out of the equipment evaluated here, the data supports the choice of A in tandem with C for automated low volume, formulation and fill. However, option A in combination with manual fill may also be considered as a low-cost, semi-automated alternative.

## Conclusion

- Option A is recommended for wash and formulation followed by manual fill to achieve high cell recovery, cell stability and fill accuracy.
- If automation is desired, A recommended for wash and formulation followed by C for fill.
- Container C1 recommended for low volume fill.

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