Optimizing volumes during cell processing: A predictive model for immunotherapy workflows

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Abstract

Optimizing cell harvesting in a closed and automated way can help reduce the processing time for expanded cell therapy products, and deliver treatments to patient faster. Cytiva's CultureWash C-Pro application works with Sepax™ C-Pro to enable automated processing, maximize cell recovery, and minimize operator intervention while avoiding open, manual techniques.

Using a model human B-lymphoblastoid cell line 721.221 (Rutgers University; hereinafter "221s"), we hypothesized that intermediate volume would be related to cell diameter and pellet size. We tested a mathematical formula to calculate the minimum intermediate volume that applies to up to 4 billion total cells, for a final product dose of 20 mL. We also tested an optimal parameter set for the CultureWash protocol for values above this cell number.

In this study, we achieved recovery of > 88% for harvesting up to 4 billion cells using a standard parameter set, and were able to increase recovery from ~ 68% to > 86% for larger cell numbers using an optimized parameter set.

Study design: Sepax™ C-Pro setup

We used the CT-60.1 single-use kit that is compatible with the CultureWash C-Pro application. We systematically increased the intermediate volume with an increase in cell number per the predictive formula, and measured recovery and viability at each processing step.

With an average cell diameter of 221s being 16 µm, our formula predicts that roughly 2.57 mL of IV is required for every 1 billion cells including 20% extra volume as a buffer region.

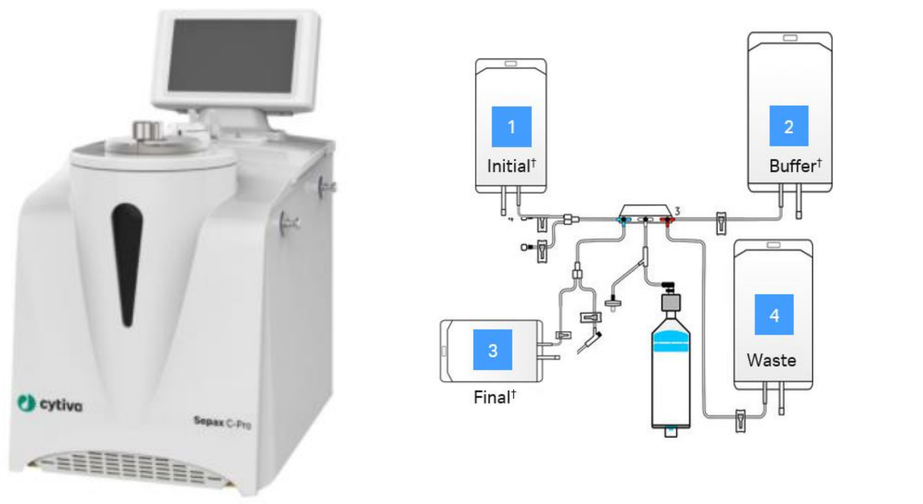

Fig 1. (Left) Sepax™ C-Pro device capable of running the CultureWash C-Pro application. (Right) Three identical samples were taken from each Sepax™ C-Pro run. Bag locations are shown for CT60.1 Kit. Users must supply bags denoted with a dagger (†) as they are not part of the kit.

Predictive formula (hypothesis)

$$IV[mL] = \frac{4\pi C(\frac{D}{2})^3}{3} * 1.2 * 1e6$$

Where:

IV = minimum intermediate volume [mL]

C = Total cell number being processed [# cells]

D = average cell diameter [m] – e.g., 16 µm = 1.6e-5 m

1E6 = volumetric unit conversion constant [cm^3 per m^3]

1.2 = 20% extra volume buffer factor

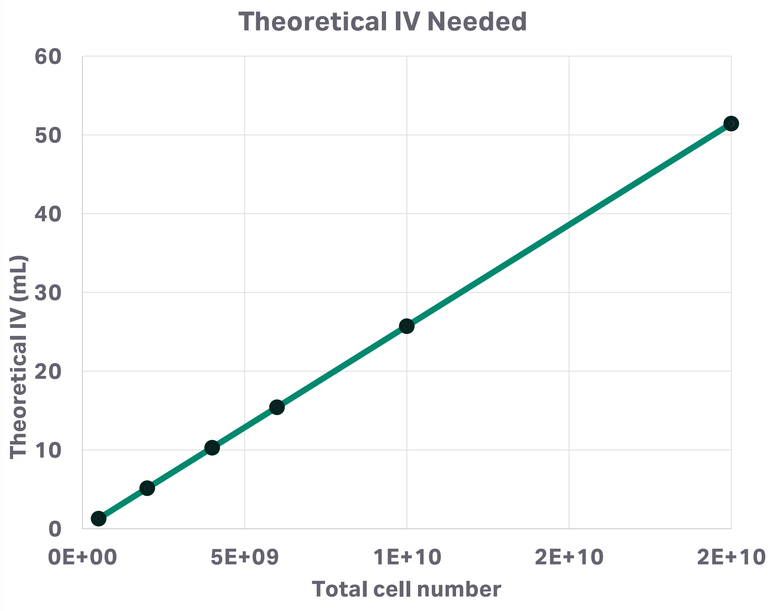

Fig 2. Predicted IV need per total cell number for cells 16 µm in diameter.

Results

Cell recovery and viability

Average recovery for the 2 and 4 billion cell conditions was greater than 88% (Fig. 2) (2B:IV5 = 90.8% +/- 0.5%, 4B:IV10 = 89.2% +/- 0.6%). The 6 billion cell condition with an IV of 15 mL had a significantly lower recovery, while using the same CultureWash C-Pro parameter set. We were able to improve the 6 billion cell condition recovery to 86.7% +/- 6.2% by decreasing the filling and extracting speed of the piston chamber. The drop in cell viability during a run was less than 1% for each run; that is, either a drop from 98% in the initial bag to 97% in the final bag, or 97% to 96%, depending on the day.

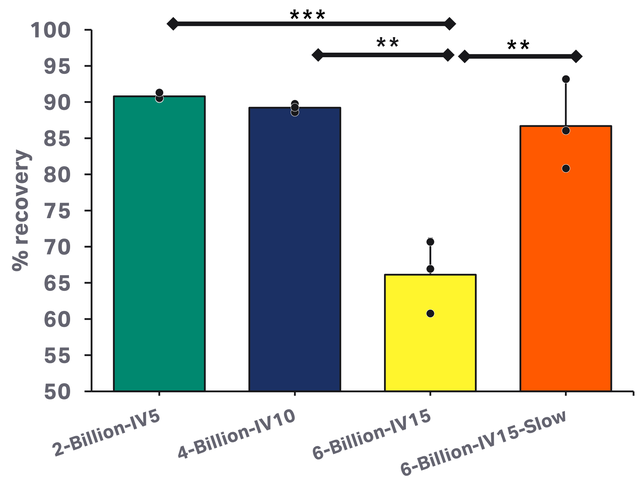

Fig 3. Recovery in the final bag for each condition. Each bar represents the average of technical triplicate +/- SD, with each replicate shown in black. Differences are only significant when compared to 6-Billion-IV15, where higher cell loss was experienced.

Decreased cell recovery due to speedy parameter set

During waste extraction of the supernatant (from concentrating the cells and washing) for the 6 billion cell condition, we observed visible cell clumps and cloudiness due to cell loss from cells shearing off the pellet.
Some potential causes for this:

- 221s are derived from a lymphoblastoid lineage and grown in suspension, so we hypothesize that they can also easily shear off
- Rapid cell diameter changes depending on the suspension medium
- Uneven distribution upon centrifugation along the periphery of the C-Pro chamber

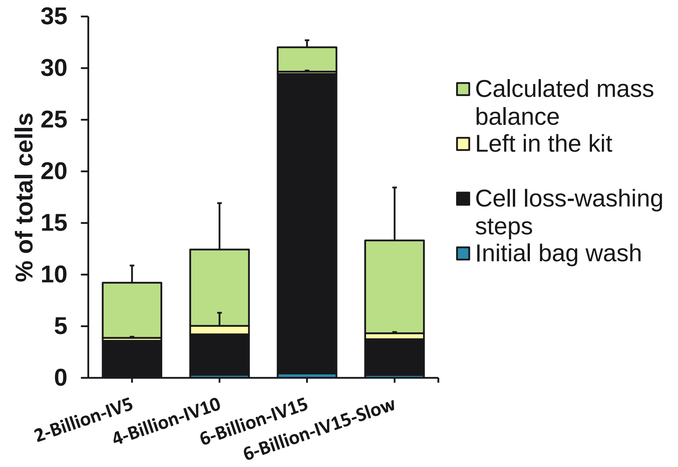

Fig 4. Breakdown of cell loss for each condition by compartment. Differences are highly significant when compared to 6-Billion-IV15, where higher cell loss was experienced during wash steps. Each bar represents the average of technical triplicates +/- SD.

Mitigation of cell loss

When starting a new cell type, there are some go-to parameters you can adjust case-by-case:

- Reduction in filling and extraction speed
- Increased sedimentation time
- Increased centrifugation speed

If maximum cell recovery is most important to your process, we recommend enabling optical detection.

Conclusions

We tested harvesting 2, 4, and 6 billion 221 cells in a final volume of 20 mL varying IV on the CultureWash C-Pro V432 according to our hypothesized mathematical model.
Our key findings:

- We achieved > 88% recovery in the linear range up to 4 billion cells for a given cell diameter of ~ 16 µm.
- For pellets greater than 4 billion cells, we recommend setting the filling and extracting speed to 30 mL/minute from the default of 120 mL/minute.
- Enable the Optical Cell Detection parameter in the CultureWash C-Pro application to automate your process and further reduce the potential for cell loss.
- Further investigation is needed to validate our model for different cell diameters and workflows.

Acknowledgments

Cytiva gratefully acknowledges the support of Dongfang Liu and Rutgers University in supplying the 721.221 cell line that we used in this work. We thank the following individuals for their help in this project; Jennifer Moseley and Celine Stoecklin.

In collaboration with

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