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Optimizing Production of Cell Banks Using an Automated Cryovial Processor

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The manual preparation of cell banks in screw-capped cryovials is a slow and labour-intensive process which, due to the sensitivity of cells to the cryopreservation solutions, limits the sizes of batches that can be produced. Manual pipetting and vial-capping tasks increase contamination risks and quality control costs, and raise the likelihood of strain and repetitive motion injuries amongst laboratory staff.

TAP Biosystems set an objective to create an automated system for reducing manual processing steps while increasing throughput, and maintaining the same or better sample quality over existing manual processes. Significant improvements have been achieved in tube processing times, reproducibility of filling volumes, and QC costs—all of which are described in this paper.

Introduction

With the ever-increasing focus on developing new biologic therapies, the need for aseptic cell preparation in cryovials is vital for the generation of high quality cell banks. However, with standard manual pipetting methods, there are a number of issues that make this problematic. Manual filling is prone to variation from operator to operator and, due to the complexity of the task, places constraints on the batch sizes that can be produced by the scientist. Additionally, the repetitive nature of the task can raise operator health concerns.

It is well known that the process of pipetting cell suspensions, whether manually or automated, can negatively impact some cell types.^[1,2]

Allowing cells to be in contact with cryopreservatives such as glycerol, dimethyl sulfoxide (DMSO), ethylene glycol, and propylene glycol for long periods of time before freezing can reduce viability in some of the well-known cell lines.^[3]

To resolve all of these problems, TAP Biosystems has identified a number of key requirements for the system.

FIGURE 1. Fill-It, an automated system capable of mechanizing the uncapping of screw-cap cryovials, dispensing cell suspensions into them, and then recapping the vials.



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Firstly, to maintain product sterility, it must be compact enough to fit into a biological safety cabinet so cells can be dispensed in an aseptic environment. To increase processing speed, the system must remove all the caps simultaneously and incorporate a pipette-free process to dispense bulk stock rapidly while assuring pre-freeze cell viability. Finally, to maintain GMP compliance, eliminate cross-contamination risks, and remove complex cleaning protocols, a fully disposable, certified tube set (including all the liquid contact components) is a must. With these parameters in mind, TAP has designed the Fill-It system (Figure 1, previous page).

This automated equipment can be installed within a

laminar airflow, biological safety cabinet and is capable of simultaneously uncapping and then recapping lids onto screw-cap cryovials from a wide range of vendors. It can also dispense liquid into multiple cryovials in parallel with minimal operator interaction. To maintain sterility and cell integrity, the system has a peristaltic pump dispensing module that uses a one-piece, sterile, disposable tube set for aseptic transfer of cells suspensions from a variety of upstream bulk stock containers into open screw-cap cryovials.

This article will describe the range of experiments conducted comparing the Fill-It system's automated performance with manual processing.

Methods of Comparison

Cell Lines Chosen

The cell lines used were Chinese Hamster Ovary (CHO) and Human Dermal Fibroblasts (HDF) cultured to $1-2 \times 10^6$ cells/mL in media prepared by a major biologics company (proprietary formulation).

Evaluating Dispensing Speed and Accuracy

To test the Fill-It system for speed and efficiency, the equipment was set up in a "dispense with suck-back" mode. Liquid dispensing speeds and precision were recorded in all rack formats. Cell dispensing accuracy measurements were recorded with cryovials arranged in racks of 96 tubes. Cycle times were recorded by filling two different vials to their maximum volumes: 5 mL in 24- and 48-place racks, and 1 mL vials in 96-place racks.

The system was set up to deliver CHO or HDF cell suspensions (1 mL , $1-2 \times 10^6$ cells/mL) into 480 tubes. After the cell bank set was generated, 96 cryovials from across the 480 tube cell bank were sampled. Throughout the series of experiments, cell numbers in each tube were

counted using a NucleoCounter® NC-100 (ChemoMetec, Gydevang, Denmark).

Evaluation results shown in Table 1 indicate that racks of 24, 48 and 96 tubes can be processed and filled in less than three minutes with a % CV of <5%. Thus, dispensing was rapid and the volume was consistent. Results in Figure 2 show that consistent cell numbers are dispensed across the cell bank using automation. The mean cell counts for CHO cells were 1.6×10^6 (with a standard deviation of 87×10^3) and for HDF cells, 1.97×10^6 (with a standard deviation of 165×10^3).

Evaluating Cell Viability of Cryopreserved Cells After Automated and Manual Vial Preparation

Viability and cell concentration of two CHO cell suspensions (750 mL) each were measured. From one CHO cell suspension, 480 aliquots (1.5 mL) were dispensed into 480 cryovials (1.8 mL capacity) using Fill-It and then frozen at -80°C to form an automatically prepared cell bank. At the same time, 480 aliquots (1.5 mL)

Vial Arrangement	24 vials	48 vials	96 vials
Dispense Precision (% CV)	<5%	<5%	<5%
Cycle Time (max volume)	135 sec	135 sec	90 sec
Dead Volume	<0.5 mL	<0.5 mL	<0.5 mL

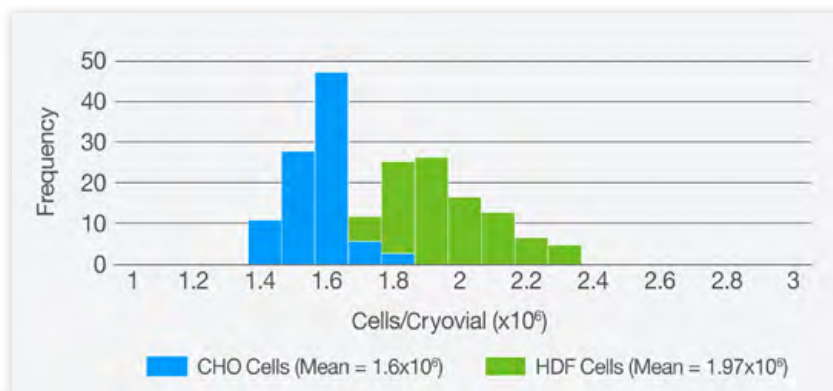


FIGURE 2. Automated dispensing of CHO and HDF cell suspensions with Fill-It.

of the second CHO cell suspension were manually dispensed by hand-pipetting into 1.8 mL cryovials. An initial comparison was made between cell concentration and viability in the bulk cell stock to that in the prepared test vials.

After preparation, the vials were frozen using a controlled rate freezer to -80°C . A sample of 25 cryovials was taken from both the automatically and manually prepared cell banks and thawed for counting. Cell counts and viability were measured immediately post-thaw. The results in Figure 3 show that automated preparation did not reduce cell numbers (<2% loss) or viability (<1% loss) compared to the original bulk cell suspension. The results

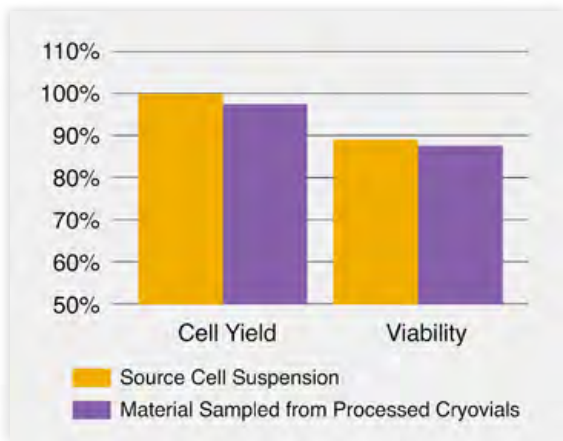


FIGURE 3. Comparison of the viability of source cell suspension with material sampled from automatically processed cryovials.

Processing Parameters	Manual	Automated
Cell Concentrations (10^6 viable cells/mL)	1.11	1.19
Cell Viabilities (%)	92.70	94.40

in Table 2 demonstrate that after a freeze/thaw cycle, the automated method produces cryovials of cells with similar or improved cell count and viability to manual methods.

From these results, it is clear that the Fill-It system provides a rapid method to produce cell banks without any detrimental impact on the cells or their viability in comparison to the standard manual processes.

Assessing the Impact of Improved Vial Filling Speed on Process Efficiency and QC Costs

Typical process steps and timings were calculated for filling 1440×1 mL cryovials using a manual process and the automated Fill-It system. Calculations were based on the processing times for the previously prepared 480-vial cryobank. By using this information, the costs associated with preparing cryovials were estimated using information provided by external cell production and supply organisations who are routinely using the Fill-It system. The analysis in Table 3 suggests that by using the automated system, a 1440 vial cell bank can be prepared in less than half the time it would take to complete this task manually—and the QC costs per vial are one quarter of the cost of manual processing (Table 3).

Processing Parameters	Manual	Automated
Equipment Set-Up (including cleaning, installation of tube set, and calibration)	0.25 hr	0.5 hr
Uncapping, Filling, and Recapping Cryovials	2.50 hr	0.50 hr
Equipment Set-Down (including cleaning)	0.25 hr	0.25 hr
Total Time Spent (set-up to set-down)	3.00 hr	1.25 hr
Number of Cryovials That Can Be Processed in 3 Hours	1440	6480
Typical QC Cost Per Processing Run	\$12 K	\$12 K
QC Cost Per Individual Cryovial	\$8	\$2

Conclusions

Rapid aseptic preparation of cryovials is vital for the generation of high quality cell banks. This study shows that automating the processes of preparing cell suspensions in cryovials using a Fill-It system provides a simple and cost-effective way of producing consistently filled vials for cell banking.

The results of this study also demonstrate that automated cryovial processing delivers cells that have

a similar viability profile to those processed manually. Additionally, automated processing of vials is very rapid (twice as fast as manual processing) and ensures cells are minimally exposed to the potentially toxic effects of cryopreservatives such as DMSO. The automated peristaltic pump used by the system can provide more reproducible processing than is possible with manual methods. It also has the potential to produce more

consistently performing cell bank lots with lower rates of failure than hand-vialed preparations due to contamination, poor viability, and low cell concentrations.

Using automated processing of cell suspensions is therefore ideally suited to use in GMP-regulated environments. Examples include the preparation of: 1) master and working cell banks for biologics research, development, and production; and 2) multiple dose aliquots of allogeneic or autologous products for cell-based therapies. In addition, the precision of the dispensing means that the automated system could be used for a wide range of other applications where accuracy is required to fill tubes with valuable, hazardous or thermally labile reagents and high viscosity solutions.

As well as providing precision, the efficiency analysis in this paper suggests that by automating the uncapping, filling, and recapping processes involved in generating a cell bank, a 1440 vial cell bank can be prepared in less than half the time it would take to complete this task manually. Importantly, by increasing the overall batch size, QC costs for a large cell bank are estimated to be 75% less than with manual processing. The faster, more efficient processing offered by automation allows high quality

cell banks to be created in a shorter period of time leading to cost savings and a rapid return on investment.

In summary, automated processing and filling of cryovials offers the potential to rapidly generate large cell banks of highly viable cells. Incorporating the Fill-It system does not require infrastructure changes, major capital investment in equipment or staffing, yet could enable significant savings on QC costs. This makes TAP Biosystems' technology ideally suited for economically generating cell banks where cell quality is critical for obtaining optimum research results or clinical outcomes.

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