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# Facilitating Production of Cell Banks Using an Automated Cryovial Dispenser

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## Introduction

The rapid aseptic preparation of cryovials is vital for the generation of high quality cell banks. Issues exist with standard manual methods as they are prone to variation, place constraints on batch sizes, and raise lab staff health concerns due to the repetitive nature of the tasks.

These can be overcome by the application of a simple bench top automated cryovial processing system - Fill-It. This system is capable of decapping, dispensing and capping multiple cryovials in parallel with minimal operator interaction. This poster describes the tests that have been conducted to show that the system can fill cryovials with material of high quality, viability and improved consistency in shorter processing times. This creates the potential to increase batch sizes and dramatically reduce QC costs.


**Figure 1: Fill-It. An automated system capable of dispensing cell suspensions into screw-cap cryovials.**

## System Description

The Fill-It system (Figure 1) is designed to be installed within a laminar airflow, biological safety cabinet to maintain an aseptic environment during operation. It consists of 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. A transfer mechanism moves racks of cryovials between the dispensing module and decapper/capper unit.

The decapper/capper unit removes, retains, and replaces the screw-caps onto the cryovials and there is facility for vacuum extraction to further reduce the presence of particulates. Fill-It provides consistent high-throughput dispensing into racks of 24, 48, 96 screw-cap cryovials from a wide selection of vendors and cryovial dimensions (Table 1). This allows operators to utilize pre-existing cryovials and not have to evaluate or change to a new format.

The Fill-It system and tube sets are both suitable for use within GMP environments subject to suitable validation testing.

**Table 1: Screw-cap cryovials from multiple suppliers and holding a range of volumes can be processed by Fill-It units.**

| Vendor | Rack Format | | |
|---|---|---|---|
| | 24 vials (1 - 5 mL) | 48 vials (2 - 5 mL) | 96 vials (0.5 - 1 mL) |
| Nunc | ▪ | ▪ | ▪ |
| Matrix | | | ▪ |
| Nalgene | ▪ | | |
| Corning | ▪ | | |

## Evaluation of Dispensing Speed and Efficiency

The system can be set up to dispense a wide range of volumes and incorporates liquid handling features that minimize the likelihood of contamination and foaming.

Cryovials can be generated immediately after preparation of cells in cryopreservative. To show consistency of cell dispensing 1 mL of CHO or HDF cell suspensions were dispensed into 480 x 1 mL cryovials. 96 cryovials from across the cell bank were sampled and cell counts performed.

Cell counts for CHO cells were (Mean = $1.6 \times 10^6$, $\sigma = 87 \times 10^3$) and for HDF cells (Mean = $1.97 \times 10^6$, $\sigma = 165 \times 10^3$) (Figure 2). Processing of vials is very rapid (Table 2) and ensures cells are minimally exposed to the potentially toxic effects of cryopreservatives, such as DMSO. For expensive cells, a de-prime facility enables solutions to be transferred back to the upstream bulk stock container, minimizing dead-volume or wastage.

In addition to cell suspensions, the system can also be used to dispense reagents, diluents, buffers and viscous solutions, such as 20% Glycerol, making it ideal for a broad range of biologics applications, including:

- Production of master and working cell banks for biologics cell line development.
- Production of cell banks to support drug discovery research applications.
- Production of pre-packaged standards and reagents for research and development.
- Production of multiple dose aliquots of single patient products for cell based therapies.

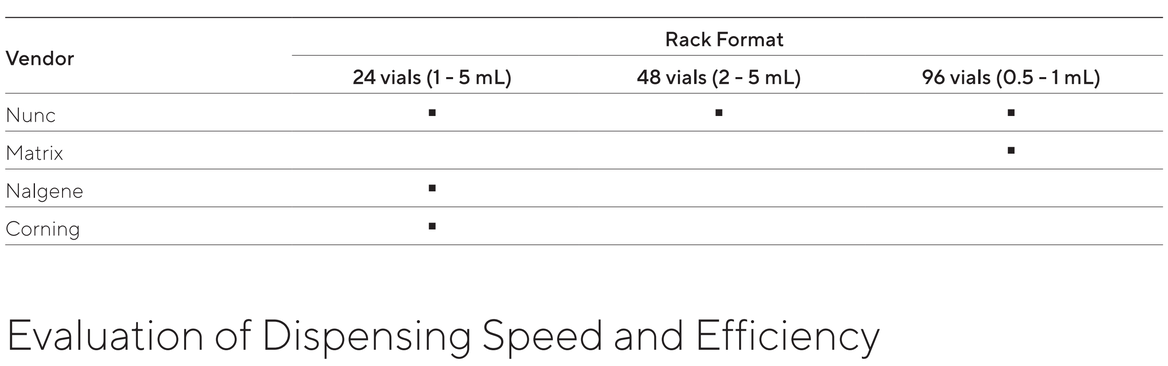


**Figure 2: Consistency of cell dispense for CHO and HDF cell suspensions.**

**Table 2: Dispense precision and speed using racks containing 24, 48, or 96 screw-cap cryovials.**

| Parameter | Rack Format | | |
|---|---|---|---|
| | 24 vials | 48 vials | 96 vials |
| Volume range | 1 - 5 mL | 2 - 5 mL | 0.5 - 1 mL |
| 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 |
| Pipetting mode | Multi-dispense with suck-back | | |

## Evaluating Cell Viability after Processing with Fill-It

Testing has shown that processing with Fill-It causes minimal trauma to cells. A comparison was performed to compare the viability (measured using a NucleoCounter) of source cell suspension with material sampled from processed cryovials. This demonstrates that Fill-It prepares cells without a significant reduction in cell number (<2% loss) or viability (<1% loss) (Figure 3). It is also predicted that cells from high quality and high viability cell suspensions are more likely to be tolerant of the cryopreservation process when carried out using Fill-It.

Table 3 shows a post cryopreservation comparison between manually prepared cryovials and those prepared using Fill-It. 1.5 mL of CHO cell suspension was dispensed into 480 x 1.8 mL cryovials and cryopreserved. Control cryovials for comparison were prepared manually. 25 cryovials from across the cell bank were revived and cell counts and viability measured immediately post-thaw demonstrating that Fill-It prepares cryovials with similar cell count and viability to manual methods.

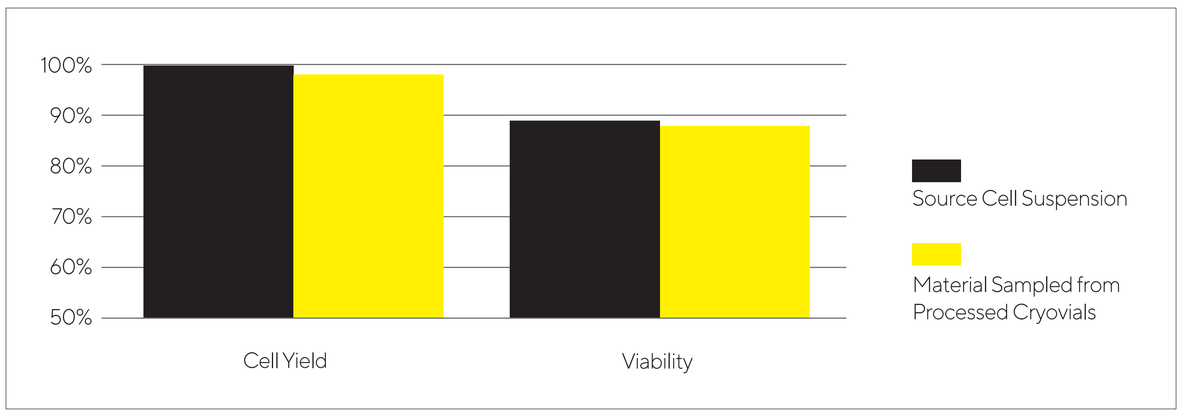


**Figure 3: Comparison of the viability of source cell suspension withmaterial sampled from processed cryovials.**

**Table 3: Post cryopreservation comparison between manually prepared cryovials and those prepared using Fill-It.**

| Parameter | Processing Method | |
|---|---|---|
| | Manual | Fill-It |
| Cell concentration ($10^6$ viable cells/mL) | 1.11 | 1.19 |
| Viability (%) | 92.7 | 94.4 |

## Assessment of the Impact of Improved Vial Filling Speed on Process Efficiently and QC Costs

A key limitation on the size of a batch of cryovials for cell banking is how much cell suspension can be processed before the quality is unduly affected by the toxic effects of cryopreservative. Therefore the time-consuming nature of manual dispensing cell suspensions into cryovials can limit the size of the cell bank produced from a single batch of cells. An analysis was performed to examine the comparative efficiency of cryovial preparation using manual and automated processes. Typical process steps and timings were determined for filling 1440 x 1 mL cryovials using a manual process and using Fill-It. The results showed that Fill-It can process the cryovials in a significantly shorter period of time.

The faster, more efficient processing offered by Fill-It allows cell banks to be created in a shorter period of time (Table 4). This also leads to the possibility of creating larger cell banks within the safe time limit for cell exposure to cryopreservation buffers. The production of a cell bank within a single process rather than multiple smaller runs means that cell bank QC costs are reduced leading to significant cost savings and accelerated return on investment.

**Table 4: Analysis of the efficiency of cryovial preparation showing potential QC cost savings.**

| Processing steps to deliver 1440 x 1 mL cryovials | Manual time (hours) | Using Fill-It time (hours) |
|---|---|---|
| Set-up equipment (including cleaning, installation of tube set, calibration) | 0.25 | 0.5 |
| Uncap, fill, recap cryovials | 2.5 | 0.5 |
| Set-down equipment (including cleaning) | 0.25 | 0.25 |
| Summary | 3 | 1.25 |
| **No. of cryovials that can be prepared within a 3 hour window** | **1440** | **6480** |
| Typical QC cost per processing run | $12k | $12k |
| QC cost per cryovial | $8 | $2 |

## Conclusion

The rapid aseptic preparation of cryovials is vital for the generation of high quality cell banks. Automating the preparation of high quality cell cultures in cryovials for storage within cell banks using Fill-It provides a simple and cost effective way of:

- Improving process efficiency with minimal change in process method
- Providing a significant reduction in QC costs
- Allowing cell bank size to scale up to match demand
- Producing high quality and consistent cell banks with similar viability to manual methods
- Reducing the dependence on labour to perform repetitive and stressful tasks.