Simplifying Progress

Evaluation and Commissioning of an Automated Cryovial Dispensing System in a GMP Cell Culture Facility

Dave Thomas, Miriam Foster, Stephen Guy

Sartorius, Royston, Herts, UK. November 2016
Royston-Info@sartorius.com

Overview

Cell banks are a vital part of many research, drug discovery and manufacturing processes. The main bottleneck in generating cell banks is dispensing cell suspensions into cryovials as quickly as possible. Performing this task manually is time consuming, can limit the size of cell banks, and put users at risk of occupational injury. Fill-It is an automated cryovial dispensing system designed to address these limitations.

Introduction

Fill-It is a bench-top system designed to allow the rapid decapping, dispensing and recapping of cryovials in an automated way and minimizes the time cells are exposed to cryoprotectant while also preventing users from performing repetitive tasks. Prior to introducing Fill-It into a GMP cell culture facility with cell banking responsibilities the system was subject to both an initial evaluation study then a commissioning study before being approved for use.

1.Method

CHO cells were revived from a master cell bank and cultured in shake flasks using chemically defined animal component free (CDACF) media until a sufficient number of cells for creation of a 500 vial cell bank was achieved. Flasks were pooled, centrifuged and resuspended at 1.0x10^7 cells/mL in CDACF media containing DMSO as cryoprotectant before dispensing into cryovials using a Fill-It system located in a Class II microbiological safety cabinet. Vials were then transferred to a controlled rate freezer for cryopreservation.

Following 48 hours of storage at -130°C or below, samples from across the 500 vial cell bank were revived by rapid warming and seeding into Erlenmeyer flasks containing CDACF media and passaged over a period of 19 days to assess viability and homogeneity of propagation. Cell health at revival and each subsequent passage were assessed manually using trypan blue exclusion.

Vials from the cell bank were also tested for sterility and mycoplasma in accordance with current European, United States and Japanese Pharmacopeia and the FDA Points To Consider, 1993. Throughout the cell culture strict aseptic technque was observed and used approved traceable consumables. At critical stages, such as pooling of cell suspensions and vial filling, environmental monitoring was performed for viable/non-viable particles.

Strict aseptic technique observed throughout study

Environmental monitoring of air and surface samples at critical stages

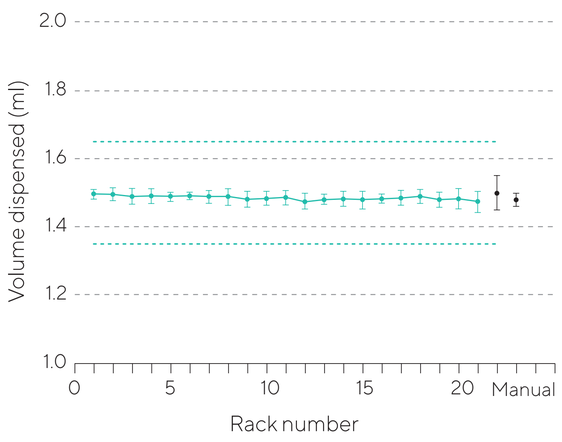
Approved traceable consumables used

Selection of representative cell line: CHO	Creation and approval of GMP documentation	Revival of cells from MCB	Expansion of cell line	Fill-It automated cryovial dispensing	Cryopreservation	Revival of cells from WCB	Viability and Homogeneity study; Sterility and Mycoplasma testing	Data analysis	Approval for Use

2. Results – Dispense Accuracy

Uniformity of vials in a cell bank is vital so the variation of dispensing was assessed. Fill-It system was calibrated for a 1.5 mL dispense volume using distilled water and then 21 racks of 24 tubes were processed sequentially. Pre-weighed tubes were removed from the racks and re-weighed to gravimetrically assess the volume dispensed assuming 1g = 1 mL.

The variation across the 21 rack Fill-It run (shown in blue) illustrates that the volumes dispensed are consistent from rack to rack and that the dispensing cassette performs within the ±10% accuracy of requested volume guarantee (shown as a blue dashed line). For comparison, cryovials were also manually prepared and weighed (shown in black). Data is summarised below.



Fill-It Dispensing	Mean ± SD	%CV
Intra-rack	1.51 ± 0.02	1.64
Inter-rack	1.48 ± 0.01	1.10

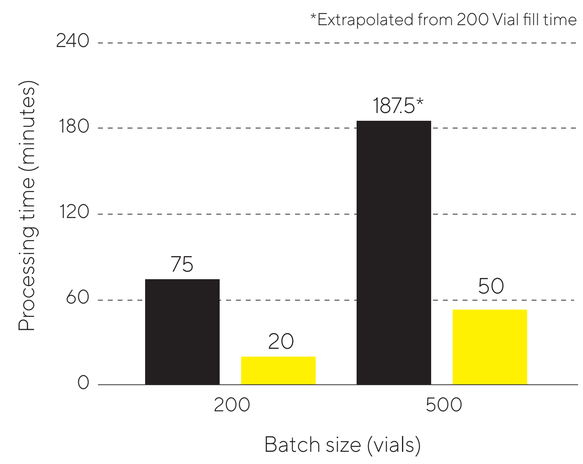
Manual Dispensing	Mean ± SD	%CV
Operator #1	1.50 ± 0.05	3.03
Operator #2	1.48 ± 0.02	1.58

3. Results – Processing Time

Manually preparing cryovials is a time consuming task and the repetitive movement can put operators at risk of occupational injury. Exposure time to cryoprotectants should ideally be as short as possible to minimize any cytotoxic effects they may have. These factors can limit the size of cell bank being created. By automating dispensing into multiple vials simultaneously it is possible to create larger cell banks in shorter periods of time.

The chart shows the time taken for the creation of two different sized cell banks both manually (black bars) and using the Fill-It (yellow bars). In both cases the Fill-It provides a significantly faster processing time such that a 500 vial cell bank can be created in less time that manual operators could create a 200 vial cell bank.

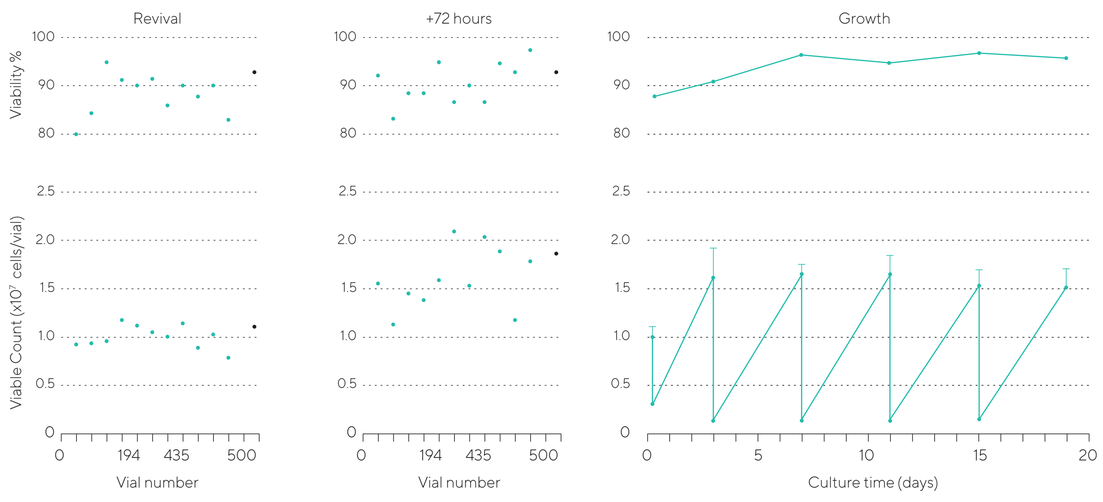
The ability to quickly process large cell banks can lead to cost savings by reducing the frequency of creating additional cell banks which involves further limit of in-vitro cell age studies, formal equivalence studies and regulatory submissions.



4. Results – Cell Recovery

Following cryopreservation sample Fill-It vials (shown in blue) were revived and cell health assessed by trypan blue exclusion (left panel) before dilution and seeding into flasks to assess the homogeneity of cell growth. 72 hours after seeding flasks were sampled to determine cell count and viability before passage (middle panel).

This process was repeated for over 19 days (right panel). Viability and viable cell counts were consistent with manually prepared vials (shown in black). 1 vial revived failed to propagate (reason unknown) and the culture was terminated after 72 hours. Data is displayed as mean ± SD, n=11.



Conclusion

- The accuracy and precision of Fill-It was equivalent to or better than manual dispensing
- Larger numbers of cryovials could be prepared in shorter periods of time compared to manual processing
- Cells prepared using from Fill-It achieved acceptable viability and viable cell concentrations at revival and were capable of propagation
- The Fill-It dispensing cassette can be approved for use as a product contact consumable and Fill-It had no adverse effect in the airflow within the Class II microbiological safety cabinet (assessed using smoke and airflow tests, data not shown)
- No microbial or mycoplasma growth could be detected in the cell bank
- Fill-It can be used in the creation of GMP cell banks