BioReliance®

Pharma & Biopharma Manufacturing & Testing Services

Technical Note

Transition to Closed Processing Systems

for the Expansion of Suspension Cells and Introduction of Ballroom Format Cleanrooms for Cell Banking

Introduction

In response to the increasing biologics manufacturing industry shift into single-use, closed system processing technologies, as well as increasing regulatory requirements to de-risk processes[1,2], our Rockville, Maryland, U.S. facility is moving routine expansion of suspension cells into a closed process format.

The closed system is designed to avoid exposure of the product or material to the room environment by connection of closed Erlenmeyer® flasks and culture components using sterile tubing and fusion systems, which will reduce contamination risk through reduction of operator intervention in the manufacturing process. Use of closed manufacturing systems allows expansion of production capacity by enabling multiple cultures to be present in the same processing area. By incorporating segregation techniques and developing cross contamination controls we are able to achieve a 'ballroom' format[3] without additional changes to the key culture systems, in terms of main product contact material during incubation.

Developed to Accomodate:

- Expansion of banks performed in closed system, utilizing shake flasks to closely align with the current expansion seed trains.
- Optimization of tubing assemblies used for cell line expansion to minimize volumetric tolerances when transferring volumes across closed junctions.
- Use of an electronic batch record and associated controls to provide traceability of the process materials and equipment used for each step of the manufacturing process.

This document outlines the process and engineering controls developed to meet the requirements for closed cell expansion of suspension cell lines.

Traditional vs. Closed Processing

Traditional methods of scaling-up cells to generate Master Cell Banks (MCBs) or Working Cell Banks (WCBs) use open culture vessels (e.g., culture flasks) with manipulations of these vessels taking place in a biological safety cabinet (BSC). This traditional method of processing requires the vessel containing the cell culture or medium to be exposed to the environment to perform manipulations such as sampling for cell counting, dilution of cells or re-seeding into a fresh culture vessel. The manual nature of these interventions, combined with open culture vessels leaves the system vulnerable to contamination through operator or environmental routes during manipulations.

In contrast, closed processing uses pre-sterilized, disposable culture labware connected through sterile means to ensure the cells remain in a protected environment during manipulations (e.g., cell expansion processes). Sterile connection systems such as tubing welds and proprietary connectors, facilitated by peristaltic pumps for liquid transfers, remove the requirement for vessels to be opened during expansion and thus significantly reduces contamination risks.

1. Annex 1 2020 draft

2. EudraLex Vol 4, Annex 2: Manufacture of Biological active substances and Medicinal Products for Human Use

3. https://www.bioprocessonline.com/doc/bioprocess-facility-design-layout-rules-and-configurations-0001

Comparability Data

In-house studies were performed to generate data on commonly banked cell types using traditional and closed culture systems. The cell lines selected for side-by-side studies were:

- Chinese Hamster Ovary (CHO) cells
- Spodoptera Frugiperda (Sf9) cells

All cell lines used were off-the-shelf cell lines optimized for antibody and protein production, using cell line supplier handling protocols for expansion criteria, seeding densities, expansion intervals, incubator settings, shaker platform settings, and recommended working volumes. To test scalability, cultures were grown until they achieved roughly 10 PDLs (Population Doubling Level) to represent the number of PDLs necessary to scale up most suspension productions.

All closed system manipulations presented below were performed in an unclassified environment and compared with the traditional splits being processed within a BSC; all media used did not contain any microbial inhibitors.

CHO Cell Line

Two vials of CHO cells were thawed and maintained in separate culture, one in traditional open Erlenmeyer® system and one in a closed equivalent. Cells in both systems were handled on the same day and each were subject to three individual splits as depicted in **Figure 1**. PDL and viability information for the same cultures and timepoints are presented in **Figures 2** and **3** respectively.

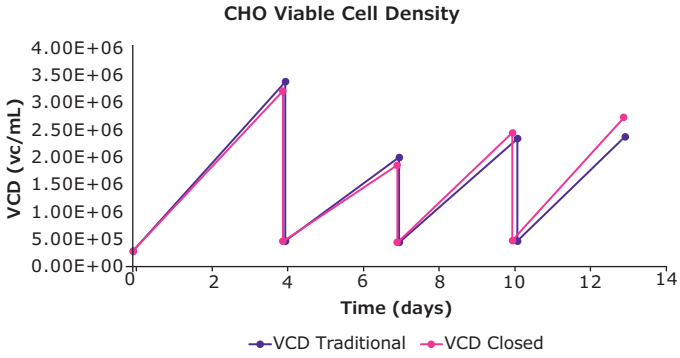

Figure 1: Comparative overview of the viable cell density for the CHO cell line study.

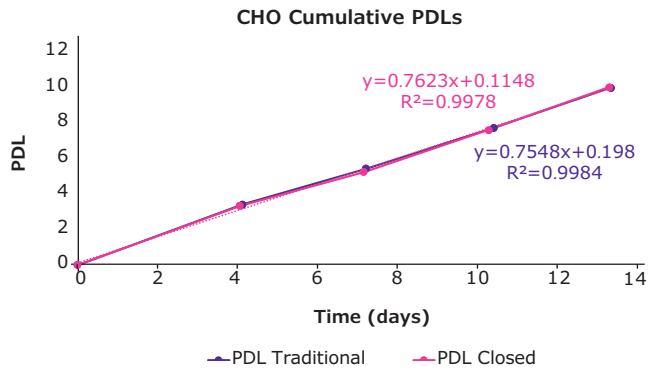

Figure 2: Comparative overview of the cumulative PDLs for the CHO cell line study.

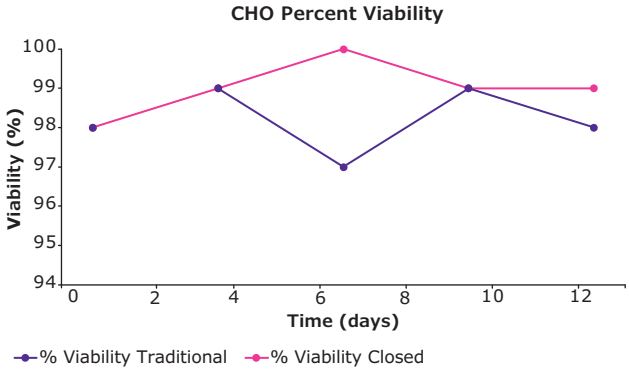

Figure 3: Comparative overview of viability fluctuations for the CHO cell line study.

Cells were kept in culture for approximately 13 days with both systems achieving more than 10.0 cumulative PDLs, which is aligned with a standard cell bank process and timeline. Culture performance in the closed system mimicked that of the open system and showed suitable for use in cell bank generation.

It was noted that the viability of the open (traditional) system differed from the closed. The closed system returned higher results at each operation on day 7 and day 13. This difference is not deemed to be significant in terms of process performance as viability has remained is considered high (>95%) throughout the process with both systems.

Sf9 Cell Line

Two vials of Sf9 cells were thawed and maintained in separate culture, one in traditional open Erlenmeyer® system and one in a closed equivalent. Cells in both systems were handled on the same day and each were subject to three individual splits as depicted in **Figure 4**, with **Figures 5** and **6** showing associated PDL and viability data, respectively.

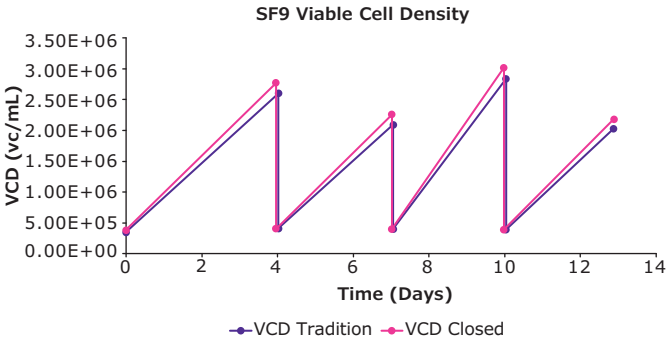

Figure 4: Comparative overview of the viable cell density for the Sf9 cell line study.

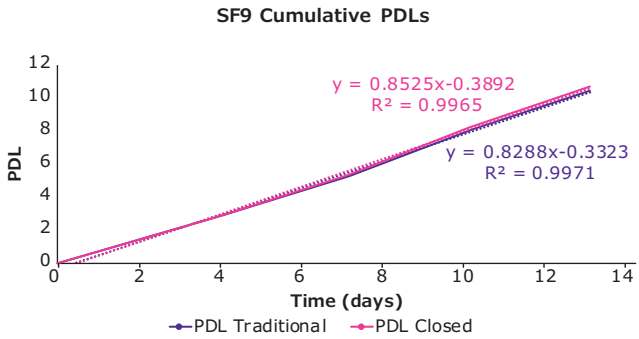

Figure 5: Comparative overview of the cumulative PDLs for the Sf9 cell line study.

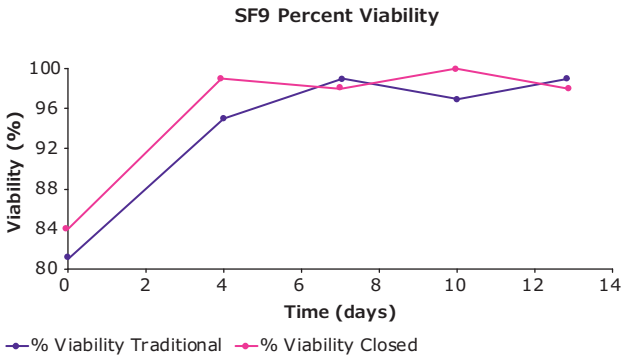

Figure 6: Comparative overview of viability fluctuations for the Sf9 cell line study.

Cells from both cultures were maintained for 13 days, after which cumulative PDL data was 10.4 for the traditional and 10.7 closed process. The minor difference in viability across the two systems is likely due to the cultures originating from two separate vials, rather than due to the culture method itself.

Ballroom Operating Principles

The introduction of closed processing and the ballroom format will increase manufacturing capacity through the ability to safely handle several cell lines in the same area (i.e., incubation and closed cell expansions).

Appropriate manufacturing controls including material traceability and segregation are key factors to mitigate cross contamination risk.

All processing steps will be documented using a custom-designed electronic batch record system which will include:

- Generating unique labels for media specific to a cell line/production run.
- Generating unique labels for each culture flask at each stage of the process.
- Scanning media or flasks (cells) barcoded label prior to use to verify the correct materials have been selected.
- Tracking material through scanning in and out of locations (incubators, etc.) in real time. This will also include verification by the electronic batch record as correct and match expected values.

The use of the electronic batch record and associated controls will provide assurance that the correct cell line and media are used for each step of the cell bank manufacture and reduce recording errors, which are more likely with manual systems.

Conclusions

The transition to closed processes for expansion of suspension CHO and Sf9 cells showed excellent comparability with traditional processing, and is expected to reach the required cell number and density for expansion and bank generation within the same timelines.

While slight perturbances in cell viability were observed, these are likely due to individual sample handling, preparation, and interpretation of the results for manual cell counting methods rather than as a reflection of the expansion method used (traditional vs. closed processing). Differences were minor (<5%) and noted at single timepoints rather than demonstrating a trend across multiple expansion processes.

Independent of the process method, all cell lines reached the preset goals of achieving 10 cumulative PDLs representative of the current range of production scale for manufacturing runs based on recovery of one client-provided vial.

By exposing the current closed processing design to an unclassified environment for evaluation purposes and comparing it to traditional open vessel and BSC-dependent operations in a controlled non-classified environment, no contamination was observed within either system. The use of an unclassified environment for expansion of cells in the closed system demonstrates the suitability of the system in maintaining an aseptic processing environment and its ability to reduce risk of culture contamination from operator or environmental sources.

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