Corning® 100 mm Aseptic Transfer Cap for 5L Erlenmeyer Flasks Provides an Aseptic Solution for Liquid Transfer and Can Be Utilized Throughout Cell Culture

Application Note

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Introduction

Closed cell culture systems play a significant role in reducing the risk of contamination during liquid handling. The Corning 100 mm aseptic transfer cap (ATC) offers a closed system solution for transferring liquid aseptically into or out of a Corning 5L Erlenmeyer flask. The ATC also contains a hydrophobic vent with 0.2 µm pore size for gas exchange, making the cap ideal for use during cell culture. The flexible tubing has a male medical plastic coupler (MPC) and is also heat sealable, allowing for a multitude of connectivity options. The vessel can be filled via gravity flow, peristaltic pump, or with negative pressure applied to the 0.2 µm vent filter of the ATC. To empty the vessel, a peristaltic pump can be used, or positive pressure can be applied to the transfer cap vent filter. Here we demonstrate the use of the ATC to seed and expand two suspension cell lines, 5/9 m alpha3-18 and Sf9 cells in the 5L Erlenmeyer flasks.

Materials and Methods

5/9 m alpha3-18 cells (ATCC® CRL-10154) were cultured in CD OptiCHO™ (Thermo Fisher 12681-011) supplemented with 8 mM L-Glutamine (Corning 25-005-CI) and 1X Hypoxanthine and Thymidine (Corning 25-047-CI). Cells were incubated in an Infors HT Multitron Pro orbital shaker at 37°C and 5% CO_2. 5/9 m alpha3-18 cells were seeded into Corning 5L Erlenmeyer flasks (Corning 431685) and 5L Erlenmeyer flasks with 100 mm aseptic transfer caps (Corning 11500) at a density of 3 x 10^5 cells/mL and a volume of 2.5 L per vessel. Cells and medium were poured directly into the 5L Erlenmeyer flasks without ATCs, and transferred into the 5L Erlenmeyer flasks with ATCs utilizing the ATC by way of a peristaltic pump at a flow rate of approximately 1L/min. Once seeded, cells were rotated at 90 rpm and counted daily, for 4 days, utilizing the Beckman Coulter Vi-CELL™ Cell Viability Analyzer. The study was repeated one additional time for a total of 5 vessels with ATCs and 6 vessels with standard caps.

Sf9 cells (Thermo Fisher 12659-017) were cultured in Sf-900™ II SFM (Thermo Fisher 10902104) at a density between 1 x 10^6 to 8 x 10^6 cells/mL in a Gallenkamp floor model orbital shaker set to 28°C. Sf9 cells were seeded into Corning 5L Erlenmeyer flasks and 5L Erlenmeyer flasks with 100 mm aseptic transfer caps at a density of 1 x 10^6 cells/mL in 3.5 L per vessel. Cells and medium were poured directly into the 5L Erlenmeyer flasks without ATCs or transferred utilizing the ATC by applying a vacuum of 2.5 inches of mercury to the ATC vent filter. Cells were rotated at 90 rpm and counted daily on the Nova BioProfile® FLEX analyzer for 4 days. The study was repeated one additional time for a total of 6 vessels per condition.

Results and Discussion

5L Erlenmeyer flasks affixed with 100 mm aseptic transfer caps allow for easy fluid transfer into and out of the vessels while also reducing the risks for contamination typically acquired by pouring. 5/9 m alpha3-18 cells seeded via an ATC at a 1L/min. flow rate from a peristaltic pump, and cultured with the ATC in place, showed equivalent high viability and cell growth throughout the 4-day culture period when compared to standard cap vessels (Figure 1). Sf9 cells seeded via an ATC with 2.5 inches of mercury negative pressure applied to the ATC vent filter, and cultured with the ATC in place, also displayed equivalent high viability and cell growth compared to standard cap vessels (Figure 2). Our data demonstrate that fluid can be transferred via peristaltic pump or by application of negative pressure without a detrimental effect to the culture, and that the cells thrive with high viability with the ATC left in place throughout the duration of culture.

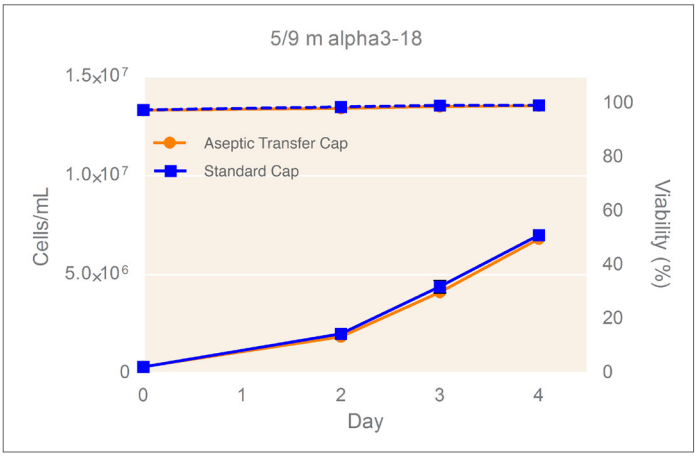

Figure 1. 5/9 m alpha3-18 cell viability was maintained above 96% regardless of whether a Corning aseptic transfer cap (ATC) or standard cap was used (right axis). Throughout the four-day culture there was no statistical difference in cell densities achieved between vessels with or without an ATC (left axis). Two-way ANOVA p>0.05, n = 5 for ATC vessels and 6 for standard cap vessels.

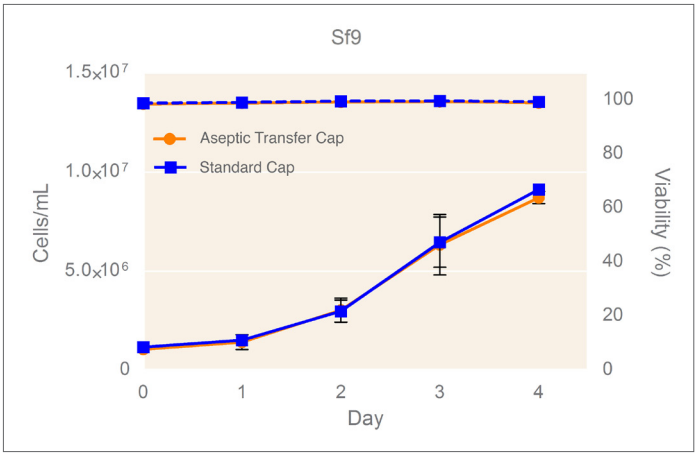

Figure 2. Sf9 cell viability was maintained above 97% regardless of whether a Corning aseptic transfer cap (ATC) or standard cap was used (right axis). Throughout the four-day culture there was no statistical difference in cell densities achieved between vessels with or without an ATC (left axis). Two-way ANOVA $p>0.05$, $n = 6$ for all vessels.

Conclusions

- Corning® 100 mm aseptic transfer caps can be utilized to aseptically transfer liquid and cell cultures into Corning 5L Erlenmeyer flasks by way of peristaltic pump or vacuum pump.
- Utilization of the ATC for filling 5L Erlenmeyer flasks has no detrimental effect on cell viability or cell growth when compared to standard cap vessels that were seeded by pouring.
- There was no statistical difference in cell densities or viability from cells cultured in 5L Erlenmeyer flasks with ATCs compared to standard 100 mm cap vessels.

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