Aseptic Fill/Finish for Flexible Cell Processing in a Cytocentric Isolator

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Abstract

Unlike a chemical drug, no final sterilization steps can be performed on a live cell product. Exposure to any cytotoxic treatment can jeopardize product quality. A major challenge for any cGMP cell production process, then, is the final fill/finish step in which live cells and sterilized packaging must be brought together without contaminating the final product. Preparing materials for cGMP-compliant clinical trials in barrier isolators can reduce bioburden risk to the highly valuable cell and tissue products[1] [2]. Unlike an open stick-built cleanroom, closed cytocentric isolators, provide ISO-5/ Class A conditions without tying into existing HVAC systems. They also maintain cells in constant and fully-monitored physiologic temperature, RH, O_2, and CO_2levels. These modular GMP systems can be quickly modified, expanded and/or replicated. This allows cell production operations to efficiently and quickly scale-up or scale-out [3]. Using a cytocentric isolator for a fill/finish process provides flexibility to a cell production operation in reducing contamination risks. In this study, we addressed sterility concerns by running mock fill/finish runs with a highly permissive bacterial broth. We performed 3 production runs, packaging the final mock cell product into sterile vials, and monitoring them for bacterial growth. Any microbial growth was detected with a color change to the test medium. We also performed environmental monitoring of the barrier isolator processing chamber with an air sampler and microbial touchplates. All positive control samples were positive for microbial growth. Negative controls and all test materials were clear. Enclosing a cell production fill/finish process inside a cytocentric GMP isolator protected aseptic products.

Background

- Cellular therapeutics and biologics cannot undergo rigorous final sterilization processes without degradation of product quality
- There is no room air the Cytocentric barrier isolator inside only tanked medical-grade gases that are continuously HEPA-filtered to ISO 5
- The modular design of the Xvivo System allows flexibility in cell therapy processing operations

Objectives

- Perform three fill/finish trials in the Cytocentric barrier isolator with TSB broth as a surrogate for a cellular therapeutic product to assess sterility
- Test for microbial contamination of barrier isolator with environmental monitoring with contact plates, an air sampler and particle monitoring

References

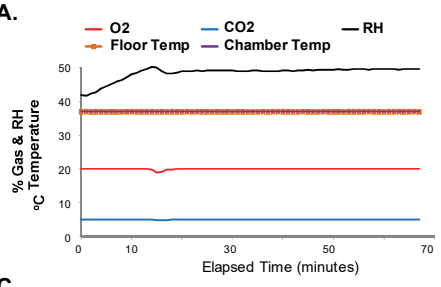
1. Wiley, L.A., et al., cGMP production of patient-specific iPSCs and photoreceptor precursor cells to treat retinal degenerative blindness. Sci Rep, 2016. 6: p. 30742.
2. Marathe, C.S., et al., Islet cell transplantation in Australia: screening, remote transplantation, and incretin hormone secretion in insulin independent patients. Horm Metab Res, 2015. 47(1): p. 16-23.
3. Yufit, T., P. Carson, and V. Falanga, Topical delivery of cultured stem cells to human non-healing wounds: GMP facility development in an academic setting and FDA requirements for an IND and human testing. Curr Drug Deliv, 2014. 11(5): p. 572-81.

Experimental Design

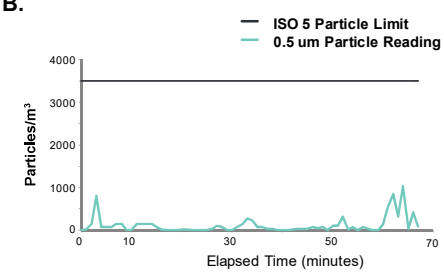
Figure 1. Experimental Set-Up
Mock fill/finish proceses were performed in a Cytocentric barrier isolator using a color-changing highly permissive tryptic soy broth (Biomerieux). Tanked, filtered medical-grade gases were used to constitute the interior air. A continuously-recirculating air cleaner (CRAC) system provided active HEPA filtration at all times. All entering materials were surface decontaminated by manual wiping with gauze dampened with SporKlenz (Steris, Mentor, OH, USA). The chamber on the left is a laminar flow hood that used HEPA-filtered room air. This chamber was utlized for disinifection and staging of all materials entering the isolator. An airlock separated this chamber from the processing chamber (just the handles of the doors are visible). There any entering air was replaced with tanked gases. All open processing was performed in the processing chamber on the right side. Mock cellular therapy products were surface disinfected, and imported into the processing chamber along with sterile vials, caps and seals. After filling, the vials were exported from the barrier isolator and incubated in a room air incubator for a minimum of 14 days. An air sampler pulled processing air across a contact plate during operations and after each trial, Rodac contact plates were used to perform environmental monitoring, testing the floor in three places, and both sets of integrated system gloves.

Results

2A.



B.

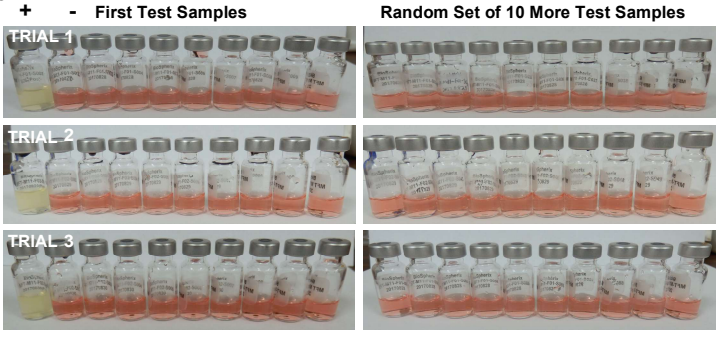


C.

	Floor Lt	Floor Mid	Floor Rt	Gloveset 1	Gloveset 2	Air Sampler	Neg Cntrl	Pos Cntrl (confl)
Trial 1	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	80%
Trial 2	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	95%
Trial 3	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	85%

Figure 2. Defined, Constant, and Aseptic ISO 5/Grade A Processing Conditions
(A) During filling processing, conditionsin the interior processing chamber of the Cytocentric barrier isolator did not vary in regard to temperatures (floor or air) or gas atmosphere. O_2 was maintained at 20% and carbon dioxide was maintained at a constant 5%. Relative humidity rose over the first 15 min of processing as liquids used in the process were opened and transfer to the final vials began. At 50%, the RH humidity control was activated and medical-grade, triple-filtered nitrogen was infused to keep the humidity at the setpoint of 50%. Oxygen dipped a bit at this point, but was quickly infused again to maintain 20%. (B) Particle levels remained below the ISO5/Grade A threshold of 3520 0.5 micron particles/m^3 at all times due to constant HEPA filtration of the air. Particles generated during motion of the gloves or opening of packaging were quickly swept away into the HEPA filters. (C) No viable microbial contaminants were detected during environmental monitoring of the isolator surfaces with contact plates nor in the atmosphere.

3A.



B.

Trial	1	2	3
# vials	100	100	100
Pos Control	+	+	+
Neg Control	-	-	-
# Pos Test Vials	0	0	0

Figure 3. Aseptic Surrogate Product Fill
Three test fill processing trials were conducted with 100 vials each. The positive and negative control vials as well as 18 test vials of each trial are shown. The positive control vials (innoculated with 0.1ml tap water outside of the barrier isolator with a needle puncture through the vial seal) were all turbid and yellow, indicating microbial growth. The negative controls and all test vials were all clear and pink, indicating that they were negative for microbial growth.

Conclusions

- The conditions within the Cytocentric barrier isolator were within ISO 5/Grade A particle level standards and held constant physiologic temperature and gas levels.
- The product surrogate showed no contamination throughout three trial production fill-finish processes of 100 vials each indicating aseptic conditions for these trials.