Aseptic Fill/Finish in a Cytocentric Isolator for Flexible Production of Cellular Therapeutics and Biologicals

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Abstract

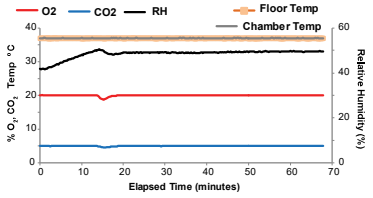
The final fill or finish step of a biologic or cell therapeutic product is a critical one for sterility. These products can't be treated harshly in a final sterilization step or the manufacturer risks severely degrading prouduct quality. The vial, the product, the lid, and the seal all have to come together under ISO 5/Class A coditions. Here we report media fill tests for this final step in the Xvivo System barrier isolators to test the sterility of conditions. The Xvivo GMP System is a flexible addition to an existing production line that does not utilize any room air, or require connections to external HVAC systems to provide an ISO 5/ Class A environment anywhere it is needed. We used a color-changing and highly permissive microbial broth to simulate a typical cell bank fill process of 100 vials. Environmental monitoring was performed during operations, using an air sampler to draw isolator air across the contact plate. We also used contact plates to assess microbial contamination in the isolator chamber itself after use. We repeated the trial six times, including the vials and plates for 14 days. We found no contamination in any of the sample products or in negative controls that had not been exposed to the isolator atmosphere. We contaminated one positive control vial and one contact plate in each fill batch and each of these was positive for microbial growth. The aseptic conditions in the Xvivo System are suitable for fill/finish steps and can safely provide a cell product or biologics manufacturer with more flexibility in capacity and operations.

Experimental Design

Figure 1. Experimental Set-Up
Mock fill/finish processing was performed in an Xvivo System barrier isolator with color-changing tryptic soy broth (Biomerieux) that is highly permissive for microbial growth. Air inside the system was from tanked, medical-grade gases and was subjected to constant HEPA filtration with a continuously-recirculating air cleaner (CRAC) system. All entering materials were manually wiped with gauze dampened with SporKlenz (Steris, USA) for surface disinfection in a laminar flow hood (LFH), the chamber on the left, with HEPA-filtered room air. Materials passed through an airlock (buffer chamber, not visible) which changed entering HEPA-filtered room air to tanked gases. All open processing was performed in the double-sided processing chamber on the right side. With prepared TSB, bagged sterile vials with caps and seals were imported. After vial filling, the vials were exported from the barrier isolator and incubated in a room air incubator for 14 days. During vial filling, an air sampler pulled processing chamber air across a contact plate. After each trial, Rodac environmental monitoring contact plates were applied to the floor in three places, and each fingertip of each set of integrated system gloves. Six separate processes were performed with 100 vials each for a total of 600 vials tested including 1 positive control injected with 0.1ml of non-sterile water for each batch.

Results

2A



B

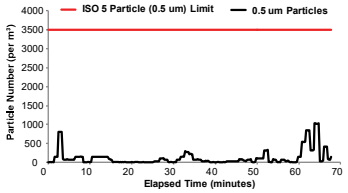


Figure 2. Optimal conditions for cell processing. (A) During vial filling, oxygen, carbon dioxide, air and chamber floor temperatures were kept constant. Relative humidity increased during the first 15 minutes of processing before the limit control of 50% was reached. At this point, dry gas was automatically infused to maintain the desired humidity level. (B) Any particles generated were swept from the chamber atmosphere by the CRAC HEPA filtration system, maintaining an ISO 5 environment.

Background

- **Cytocentric barrier isolators are a increasingly popular alternative to clean rooms for cGMP cell and tissue production.[1-3]**
- **Cellular therapeutics and biologics are far more sensitive to rigorous final sterilization steps than a chemical drug**
- **Sterile conditions in the final fill/finish step are essential for product and patient safety**
- **The Cytocentric barrier isolator excludes room air, using only tanked medical-grade gases that are continuously HEPA-filtered to ISO 5 compliant particle levels**
- **The modular design of the Xvivo System allows flexibility in cell therapy processing operations for fill/ finish as well as scale-up and scale-out**

Objectives

- **Using TSB broth with a color-changing indicator as a surrogate for a cellular therapy product undergoing a fill/finish process in the Cytocentric barrier isolator, assess sterility in six separate 100-vial test cell bank lots (n=600)**
- **Test for microbial contamination of the barrier isolator with contact plates, an air sampler and continuous particle monitoring**

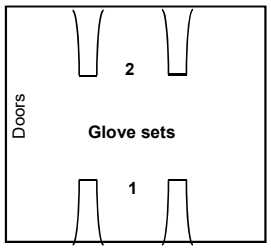
References

1. Wiley, L.A., et al., cGMP production of patient-specific iPSCs and photo-receptor 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.

3A

Lot #	1	2	3	4	5	6
Left Floor	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Middle Floor	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Right Floor	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Glove Set #1	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Glove Set #2	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Air Sampler	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
(+) Control (Confluence)	80%	95%	85%	95%	90%	95%
(-) Control	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU

B



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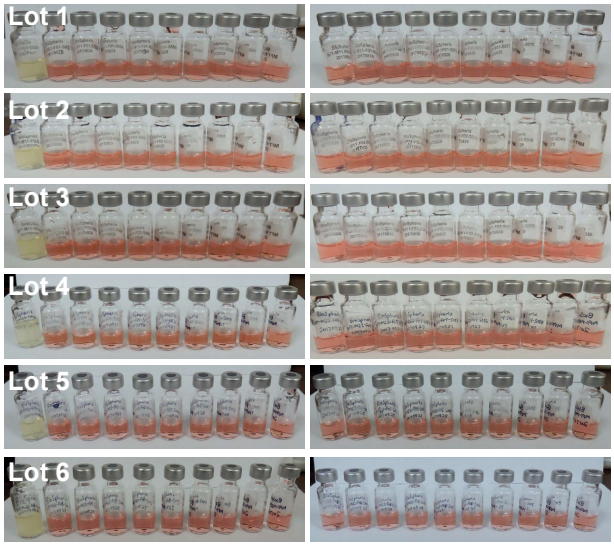

D

Lot#	Pos	Neg	Pos Control
1	0	99	+
2	0	99	+
3	0	99	+
4	0	99	+
5	0	99	+
6	0	99	+

Figure 3. The Mock Cell Product and the Chamber were Sterile During and After Vial Filling. (A-B) Surfaces monitored included fingertips on 2 sets of gloves and 3 points on the chamber floor. The chamber air was monitored using an air sampler to draw air across a touchplate during operations. Touch-plates were incubated for at least 5 days. Positive control touchplates were touched to surfaces outside of the Xvivo. Negative control plates were not exposed. Positive controls showed microbial growth, otherwise, plates were negative. (C-D) Photos of the first ten vials plus another random 10 vials of each 100 vial lot (after at least 14 days of incubation) is shown. The positive control for each lot (far left of each panel) was yellow and turbid, otherwise samples were negative for microbial growth.

Conclusions

The Xvivo System maintained aseptic conditions throughout the fill/finish step for a cell therapeutic or biologic product without compromising ideal cell conditions