Closing the Fill/Finish Step for Reducing Risks to cGMP Virus or Cell Production

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ABSTRACT

Fill/finish is an especially risky step of the production of any biological therapeutic. Absolute sterility has to be accomplished without compromising live product quality. Here we report media fill tests for this final step in the aseptic Xvivo GMP System to test the sterility of conditions. This system provides a continuously HEPA filtered ISO 5/ Class A processing space without using any room air or external HVAC systems. It can be charged with dry Nitrogen for an inert atmosphere or maintain a constant 37 C/ 5% CO2/ physiologic O2 to avoid suboptimal transients for a cellular product. Personnel, and the bioburden associated with them, are separated from the aseptic workspace by a soft, flexible glovefront. We used a highly permissive color-changing microbial broth to simulate a typical vial fill process of three batches of 100 vials each. We used an air sampler to draw processing chamber air across a contact plate during filling. Before and after each batch, we used contact plates to assess microbial contamination of probable risk surfaces like gloves, sleeves, floor, and doorknobs. We incubated all positive controls, test vials and plates for up to 14 days, evaluating them at 1, 5, 7, and 14 days. Positive control plates and vials all showed ample evidence of contamination within 5 days while none of the test samples showed signs of contamination. Environmental monitoring plates for air and probable risk surfaces also showed no signs of contaminations (<1CFU). We conclude that the Xvivo GMP System is an aseptic environment that can reduce risks for the critical fill/finish step of any cGMP viral or cellular production process.

BACKGROUND

- **Fill/Finish of live cell products is a high-risk step[1]**
- **Disinfection chemicals pose a direct risk to protein[2] and cell product quality[3]**
- **We designed a fill/finish study using a highly permissive color-changing Tryptic Soy Broth as a surrogate product**

DATAPOINTS

- **3 Trials x 100 Vials**
- **Touchplates 5 Probable Risk Surfaces Before/After Fills**
- **Touchplates in Air Sampler During Fills**
- **Conditions Monitored by the Xvivo System**

EXPERIMENTAL DESIGN

Figure 1. Experimental Design. All processes were performed in the Xvivo System which provides a completely controlled atmosphere from filtered, tanked gases. No room air is inside. The atmosphere is continuously HEPA filtered for particle control to ISO 5 levels during cell processing. The temperature of the floor and air of each chamber can be controlled to constant 37°C. The black doors cover integrated cell incubation chambers. Materials were brought into the system through the laminar flow hood on the left. Buffer chamber air locks exchange any entering room air with tanked gases before materials were imported. Before processing, probable risk surfaces inside the Xvivo and all imported materials were surface disinfected with SporKlenz RTU (Steris). Sterile vials were filled with color-changing highly permissive TSB broth (BioMerieux). After pre-process cleaning, touchplates were used to assess probable risk surfaces for microbial contamination. This was repeated before post-process cleaning. During vial filling, an air sampler was used to draw processing chamber air across a touchplate. All vials and touchplates were incubated for 14 days or 48 hrs respectively and vials were assessed at Days 7 and 14 for contamination analysis. n= 3 trials of 96-100 vials each. Positive controls were exposed to the room environment outside the Xvivo System. Negative controls were not exposed at all.

RESULTS

	Trial #1	Trial #2	Trial #3
# Vials	100	100	96
Pos. Control	+	+	+
Neg. Control	-	-	-
# Pos. Test Vials	0	0	0

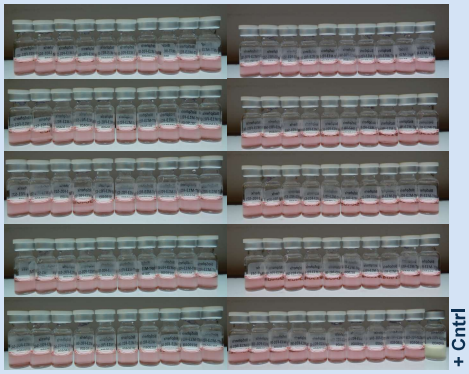

Figure 2. Surrogate Product Results. Three batches of vials were filled. 100 in Trials 1 and 2, and 96 vials in Trial 3. Vials were incubated for 14 days and photographed on Days 7 and 14. Positive control vials for each trial were yellow and turbid, indicating microbial growth. All other vials were pink and clear, negative for microbial growth. The table shows all results. Representative photos are of the vials from one trial at Day 14 of incubation.

Location	Trial #1		Trial #2		Trial #3	
	Before	After	Before	After	Before	After
Left Floor	< 1 CFU	< 1 CFU	err	< 1 CFU	< 1 CFU	< 1 CFU
Center 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
Door Knobs	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU	< 1 CFU
Gloves	< 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
Negative Ctrl	< 1 CFU		< 1 CFU		< 1 CFU	
Positive Ctrl	~90% confluent		~85% confluent		~80% confluent	

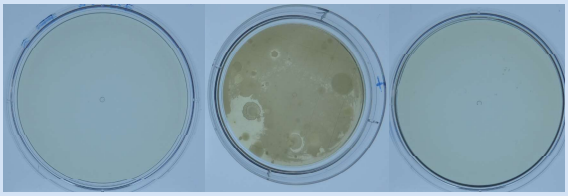

Figure 3. Environmental Monitoring. Touchplates filled with BBL Trypticase Soy Agar (BD) were touched to probable risk surfaces inside the processing chamber of the Xvivo System. Sites were chosen for one plate at each of three sites on the chamber floor, one plate for all of the door knobs on buffer chamber, one plate for the fingertips of two gloves of the glovefront, and one plate for the air sampler. The air sampler was run during the vial filling process inside the processing chamber. The positive control plate for each trial was exposed to an outer Xvivo Surface in the room. Negative control plates were not exposed at all. One pre-processing plate in Trial 2 was accidentally opened outside of the Xvivo System and was excluded from results. All test plates and negative controls were negative for microbial growth. All positive plates were positive. This shows that the chamber environment was not contaminated before or during the filling process.

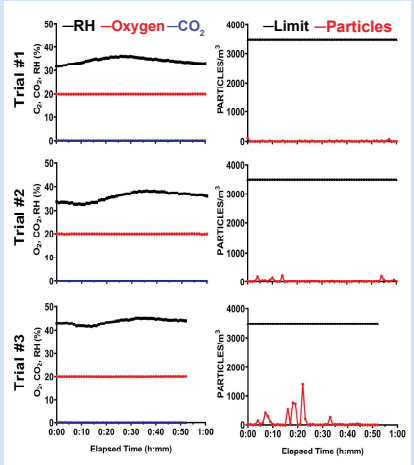

Figure 4. Atmospheric Conditions. During vial filling, the Xvivo System monitored and actively controlled chamber O2, CO2, relative humidity (RH), and particle levels. Oxygen (red line) was set to constant 20% (room air levels), CO2 (blue line) set to 0.1%. The RH limit control was set to 50% meaning that if humidity exceeded 50%, dry gases would be automatically infused. The limit control for particles (>0.5 µm) was set to 3500 0.5 micron particles (or larger) per cubic meter, the ISO 5 limit. This level was never reached during processing. The Continuously Recirculating Atmosphere Cleaning HEPA-filtration system kept particles below this limit.

CONCLUSION

The Xvivo System maintained a fully aseptic environment during vial fills.

REFERENCES

1. Reid IMM: Risk-Averse Fill/Finish Industry Embraces Change. PharmTech.com Sept., 2, 2018.
2. Wang W, Cui TY, Wang YJ, Martin-Moe S: Oxidation of protein by vaporized sanitizing agents. PDA J Pharm Sci Technol 2004, 58(3):121-129.
3. Sagripanti JL, Bonifacino A: Cytotoxicity of liquid disinfectants. Surg Infect (Larchmt) 2000, 1(1):3-14.