Considering Safety:
Evaluating Comprehensive Biodecontamination of Sensitive Equipment

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Background

Hypoxia chambers are an emerging technology designed to mimic environments, including those seen in the human body. These chambers enable control of environmental conditions within the chamber via specialized sensors and filters. The user can grow organisms under specified conditions or mimic the internal environment of the human body to test medications in the exacting way in which they will be used rather than in a static laboratory environment as is the case with standard pharmaceutical development. This precision means it is vitally important to have comprehensive contamination control of these environments. Currently employed decontamination solutions include spray and wipe disinfection, ethylene oxide, and high concentrations of hydrogen peroxide, any of which may create toxic off-gassing and residues. **Concerned that traditional high consequence chemical biodecontamination via gaseous treatment may compromise sensitive equipment, such as sensors found in a hypoxia chamber, Baker tested the feasibility and efficacy of a low level gaseous hybrid hydrogen peroxide system in decontaminating its hypoxia chamber.** This experiment was developed to establish an optimal protocol for decontamination of a three-glove hypoxia chamber, to determine if low concentration vaporous 7% hydrogen peroxide would be efficacious, and to determine if the decontamination would alter the operation of the hypoxia chamber with its delicate onboard sensors. **In essence, could a low level 7% hydrogen peroxide vapor produce sporicidal results without harming the hypoxia chamber's normal operation?**

Hypoxia Chamber and Biodecontamination Device

Methods

The biodecontamination system chosen for this study was a closed-loop device, which conditions containment enclosures, delivers a vaporous low level 7% hybrid hydrogen peroxide to the space, and extracts vapor at the end of each cycle. The modular biodecontamination system attaches to the hypoxia chamber via inlet and outlet ports, allowing the chamber to remain closed and preserving the integrity of the chamber's internal environment during treatment.

Eight individual test rounds were performed on the hypoxia chamber. Five tests were used to establish the decontamination system cycle within the chamber, with the remaining three validation tests used to determine sporicidal efficacy of that cycle.

Testing Procedure

- Hypoxia chamber was set to 30% Rh and interlock door opened
- Biological indicators in Tyvek/Tyvek pouches and chemical indicators were placed in identified locations (Diagram 1)
- Decontamination system was connected to hypoxia chamber via cam locks and hoses
- Decontamination system cycle was initiated
- Decontamination system progressed through sampling and conditioning phases
- Decontamination system Extraction Pod was inserted after pulse injection phase
- Following cycle completion, biological indicators were placed into tryptic soy broth, and incubated
- Biological indicators were observed at 24 hours and again after 4 days and results recorded.

Materials

- Hypoxia chamber, SCI-tive model, BAKER Ruskinn, Bridgend, Wales, UK
- TRINITY™ system, CURIS System, Oviedo, FL
- Biological Indicators (39), Mesa Labs, 2.0 x 10^6 Tyvek®/Tyvek® D-value of .7 minutes
- CURoxide™ solution, EPA registration #93324-1, CURIS System, Oviedo, FL
- Chemical Indicators (39), CURIS System, Oviedo, FL

8 Total Tests
3 Validation Runs
39 Biological Indicators

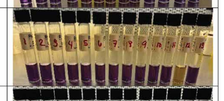
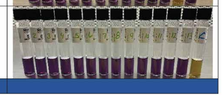
Date	Total # BI(s)	# BI(s) Passed	Location of Failed BI	Image
9/30/2021	13	13	n/a	
9/30/2021	13	12	#12	
10/1/2021	13	13	n/a	
Total	39	38		

Table 1: Biological Indicator Results

Validation

For validation of successful 6-log reduction, a minimum of 13 locations in the hypoxia chamber were tested, with one biological indicator (BI) of *Geobacillus stearothermophilus* (2.0 × 10^6 organisms) on a steel spore carrier and one H_2O_2 chemical indicator (CI) placed at each location. At the end of each treatment cycle, BIs were aseptically processed, incubated at 55 degrees Celsius, and observed for 24 hours and again at 4 days.

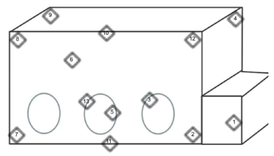
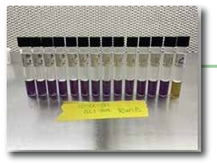


Diagram 1: BI & CI locations in the Hypoxia Chamber

1) Insider Interlock
2) Lower Front Right Corner
3) Lower Back Right Corner
4) Upper Back Right Corner
5) Center on Workspace
6) Left Back Wall
7) Lower Left Front Corner
8) Upper Left Front Corner
9) Upper Left Back Corner
10) Under Ceiling - Center
11) Under Work Surface - Center
12) Upper Right Front Corner
13) Under Work Surface – Back Wall



Results

A total of 39 biological indicators were challenged over three test cycles, with 38 of the 39 indicators demonstrating a 6-Log sporicidal inactivation. Over three test cycles, one indicator demonstrated growth, possibly due to contamination caused by external fans powering on before BI collection, which was a deviation from test parameters. Subsequent testing adhering to test parameters proved successful in all locations (Table 1). Throughout testing, observations showed the hypoxia chamber maintaining normal operation of its sensitive onboard sensors, which are critical to maintaining specific environments within its contained enclosure.

Conclusion

Overall, successful results show that the low level 7% hybrid hydrogen peroxide vapor-only system is an effective tool for biodecontamination of closed-loop enclosures, including hypoxia chambers. Normal operation of the hypoxia chamber following eight decontamination cycles with the 7% hybrid hydrogen peroxide system demonstrated a potential for safer use on these sensitive environments over traditional methods and may demonstrate a significant advantage over higher concentration solutions where delicate sensors are involved. This, coupled with repeated validatable 6-log sporicidal efficacy, demonstrates the TRINITY device by CURIS system is a viable vaporous decontamination system for use with hypoxia chambers.

Further Study

Continued ongoing experiments are underway to ensure comprehensive kill and thorough compatibility of the hybrid hydrogen peroxide system and the hypoxia chamber. The outcome of this ongoing work will further clarify the suitability of hybrid hydrogen peroxide for hypoxia chambers.