November 2020 to January 2021

Biodecontamination of Cleanrooms using Hybrid Hydrogen Peroxide™ Technology

Synopsis

This study shows the comprehensive biodecontamination of a mobile cleanroom using a Hybrid Hydrogen Peroxide™ vaporous delivery system. It demonstrated a repeatable, validatable, 6-log reduction throughout the entire facility (hazardous, ante rooms, non-hazardous, and common rooms), including equipment (pass throughs, BSCs, and work benches).

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Background

Cleanrooms are controlled environments that are commonly used for manufacturing anything from semi-conductors to medical or pharmaceutical devices. They are highly specialized to filter out particulates and microbes that can impair the integrity of what is being produced and endanger the safety of staff.[1] Contamination control within these enclosures is a never-ending battle for facilities, so strict GMP and FDA regulations that determine how the room should be decontaminated must be followed to ensure there is no contamination. While it is not required, it is highly recommended that a sporicidal disinfectant be used on sterile surfaces. The most common sporicidal disinfectants being used in the cleanroom pharmaceutical industry include vHP, peracetic acid, and other disinfectants that use active oxygen.[1]

The various levels of cleanrooms (ISO 1-9; Class 100-10000; A-D) dictate the regulation of particles per cubic meters of air, air changes per hour, and the necessity of extensive filtration systems. ISO 1 is considered the "cleanest" and ISO 9 is considered to be the "dirtiest" cleanroom category.[2] On average, ISO 7 and 8 are the most common cleanrooms installed. Cleanrooms ISO 4-8 employ c-GMP standards, while higher grade cleanrooms require all disinfectants to be supplied sterile or have the ability to be sterile filtered by the cleanroom operators. Disinfection is required when there is maintenance work being completed or if there is a filter failure to ensure cleanliness of the space. A biodecontamination system with the ability to provide a 6-log repeatable reduction of surfaces as well as within filters could be advantageous to these cleanroom environments.

Traditionally, decontamination processes include multiple steps and are wrought with opportunity for potential contamination. Some practices include sweeping, mopping, and wiping surfaces.[3] In certain cases, the very detergent used to clean or disinfect these enclosures must include the added step of wiping down equipment due to potential corrosion or chemical residue. It can also be time-consuming and pose tangible risks to staff. Some additional risks include human error by missing certain areas or even causing contamination. In contrast, CURIS' automated Hybrid Hydrogen Peroxide™ (HHP™) delivery system has proven able to quickly ensure all surfaces that air touches are treated in a wide variety of enclosed environments. This HHP™ device is known to achieve a 6-log reduction[4] without compromising the integrity of materials in the room and is relatively safe compared to caustic chemicals like chlorine dioxide, carcinogens like formaldehyde and ethylene oxide, or high consequence chemical concentrations of 35-59% hydrogen peroxide. When these cleanrooms are down for maintenance, biomarkers are placed and those tested must be an "acceptable value" in order to pass the environmental testing (USP 797 guidance says CFUs under 1 are acceptable).

Additionally, when employing gaseous hydrogen peroxide for biodecontamination, it is common to employ biological indicators with a population of $1x10^6$ of *Geobacillus stearothermophilus*, identified by BMBL as sterilization markers. To determine if this HHP™ system could provide an effective improvement to currently employed technologies, one CURIS device was challenged to treat the entire mobile cleanroom with all its separate spaces, embedded equipment, and HVAC filtration system simultaneously. This study shows the results of implementing CURIS' 7% hybrid hydrogen peroxide system within a cleanroom environment.

Materials

- Germfree Standard Mobile Cleanroom: ~3,000 ft3
- *Geobacillus stearothermophilus* biological indicators, MesaLabs: Apex Discs, Lot# AH-117, Strain 12980, population 2.0 x 10^6, Tyvek/Tyvek, D-value of 0.7 minutes
- Modified tryptic soy broth media
- CURIS Core (1)
- Box fans (2)
- CURoxide™ 7% hydrogen peroxide
- H_2O_2 Chemical Indicators (3M)
- Anemprobe for detailed H_2O_2 tracking
- iPad (for use of CURIS Decon App)
- 3rd Party Sampling
    - USP 797 Culture, Surface, Fungal Counts with ID: SOP 3.9
    - USP 797 Culture, Surface, Bacterial Counts with ID: SOP 2.23
    - USP 797 Culture, Air, Bacterial Counts with ID: SOP 2.2

Methods

Prior to the final biodecontamination tests, a series of optimization tests were completed to finalize a procedure for biodecontamination of this particular mobile cleanroom. One CURIS device was used to fully decontaminate the entire ~3,000 ft^3 area, including embedded equipment and the cleanroom's integrated HVAC. The airlock doors were opened, and fans were placed (one in each ante room) to create additional airflow to reach all corners of the space. The cubic footage was entered into the device with a boost of 30% to account for the biodecontamination of the cleanroom HEPA filtration system and equipment. It was determined that the airlock doors would be disarmed and both sides could remain open since the space was considered "down for maintenance."

After establishing the optimized protocol, three repeated biodecontamination tests were completed on three separate occasions from November 2020 through January 2021. The starting relative humidity, temperature, and biological and chemical indicator locations were recorded. An Anemprobe was used to track more detailed H_2O_2 concentrations for the last two runs.

Two fans were placed within the facility to ensure circulation of HHP™ treatment throughout all rooms in the mobile cleanroom and its filtration system simultaneously and decrease the biodecontamination time. Figure 1 shows the horizontal airflow throughout the space and the location of the device and fans.

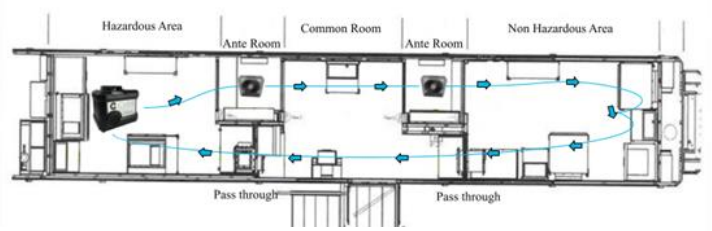

Figure 1. Mobile cleanroom horizontal airflow.

Trials 1-3: Biological and Chemical Indicator Locations

For each of the first three tests, prior to the activation of the CURIS device, biological indicators (BIs) and chemical indicators (CIs) were placed in varying locations to validate complete biodecontamination of the space (Figure 2).

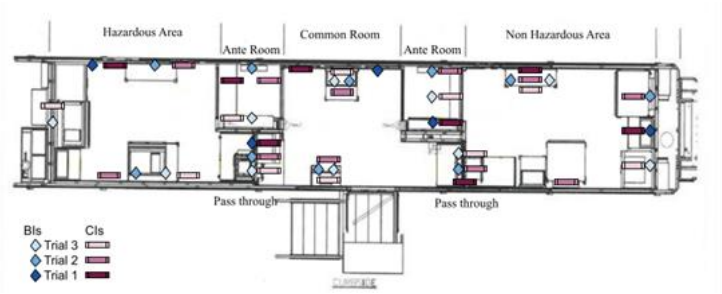

Figure 2. Mobile cleanroom biological indicator and chemical indicator placement.

Upon completion of the biodecontamination cycle, BIs were aseptically collected, incubated, and results were recorded after a period of 7 days. Their placements are detailed in Tables 1-3 below.

Table 1. Trial 1 chemical indicator (CI) and biological indicator (BI) placement.

Trial 1	CI Placement	BI Placement
	Right Wall – Hazardous	Left Right Wall – Hazardous
	Pass Through – Hazardous	Pass Through – Hazardous
	Ante – Hazardous	
	Side Wall - Common Space	Side Wall – Common Space
	Pass Through - Nonhazardous	
	Ante Room – Nonhazardous	Ante Room – Nonhazardous
	Table – Nonhazardous	
	Back Wall – Nonhazardous	Back Wall – Nonhazardous

Table 2. Trial 2 chemical indicator (CI) and biological indicator (BI) placement.

Trial 2	CI Placement	BI Placement
	Rear Wall - Hazardous	Rear Wall - Hazardous
	Rear BSC - Hazardous	Rear BSC - Hazardous
	Pass Through - Hazardous	Pass Through - Hazardous
	Ante Room - Hazardous	Ante Room - Hazardous
	Under Table – Common Room	Under Table – Common Room
	Work Bench – Common Room	Work Bench – Common Room
	Pass Through – Nonhazardous	Pass Through – Nonhazardous
	Ante Room – Nonhazardous	Ante Room – Nonhazardous
	Under Table – Nonhazardous	Under Table – Nonhazardous
	Rear Wall – Nonhazardous	Rear Wall – Nonhazardous
	Rear BSC – Nonhazardous	

Table 3. Trial 3 chemical indicator (CI) and biological indicator (BI) placement.

Trial 3	CI Placement	BI Placement
	Rear Wall - Hazardous	Rear Wall - Hazardous
	BSC (Off) - Hazardous	BSC (Off) - Hazardous
	Pass Through - Hazardous	Pass Through - Hazardous
	Ante Room - Hazardous	Ante Room - Hazardous
	Under Table – Common Room	Under Table – Common Room
	Work Bench – Common Room	Work Bench – Common Room
	Pass Through – Nonhazardous	Pass Through – Nonhazardous
	Ante Room – Nonhazardous	Ante Room – Nonhazardous
	Under Table – Nonhazardous	Under Table – Nonhazardous
	Rear Wall – Nonhazardous	Rear Wall – Nonhazardous

Trials 4-10: 3rd Party Validation

An additional seven tests were conducted by the staff using 3rd party validation. Following similar protocols, numerous locations throughout the cleanroom were tested for fungi and bacteria via air sampling and surface swabbing. Sample plates were then incubated and evaluated for growth, and results were reported by a 3rd party.

Results and Conclusions

Using the protocols established in this study, CURIS' HHP™ device achieved a consistent 6-log sporicidal efficacy within the mobile cleanroom, including within all equipment and the filtration system simultaneously. There was a 100% passing rate on all 25 biological indicators placed in 11 distinct locations throughout the entirety of the space for Trials 1-3. The results are listed in Table 4 below.

Table 4. Biological indicator results.

Trial 1	P		P		P				P		P
Trial 2	P	P	P	P		P	P	P	P	P	P
Trial 3	P	P	P	P		P	P	P	P	P	P

The air (A) and surface (S) sampling results for Tests 4-10 showed consistent success with 100% passing for all locations tested in compliance with USP 797 and CAG-009 guidance (Table 5).

Table 5. 3rd party validation results of air and surface sampling.

#	Sample Location	Sample	Class	Pass	Fail
1	Negative Control TSA Lot #139687	A	N/A	P	
2	Negative Control SDA Lot#139301	A	N/A	P	
3	Positive Control TSA Lot#139687	A	N/A	P	
4	Positive Control SDA Lot#139301	A	N/A	P	
5	LF S/N 65-15-BH-19941	A	5	P	
6	LF S/N 65-15-BS-19941	A	5	P	
7	LF S/N 35-15-BVBI-16030	A	5	P	
8	LF S/N 35-15-BVBI-16030	A	5	P	
9	BSC S/N 35-15-BR2-17550	A	5	P	
10	BSC S/N 35-15-BR2-17550	A	5	P	
11	Non-Haz Buffer Room	A	7	P	
12	Non-Haz Buffer Room	A	7	P	
13	Non-Haz Gown Room	A	8	P	
14	Non-Haz Gown Room	A	8	P	
15	Haz Buffer Room	A	7	P	
16	Haz Buffer Room	A	7	P	
17	Haz Gown Room	A	7	P	
18	Haz Gown Room	A	7	P	
19	Non-Haz Pass-Thru	A	7	P	
20	Non-Haz Pass-Thru	A	7	P	
21	Haz Pass-Thru	A	7	P	
22	Haz Pass-Thru	A	7	P	
23	Ante Room/Office	A	8	P	
24	Ante Room/Office	A	8	P	
25	LF S/N 65-15-BH-19941	S	5	P	
26	LF S/N 65-15-BH-19941	S	5	P	
27	LF S/N 35-15-BVBI-16030	S	5	P	
28	LF S/N 35-15-BVBI-16030	S	5	P	
29	BSC S/N 35-15-BR2-17550	S	5	P	
30	BSC S/N 35-15-BR2-17550	S	5	P	
31	Non-Haz Buffer Room	S	7	P	
32	Non-Haz Buffer Room	S	7	P	
33	Non-Haz Gown Room	S	8	P	
34	Non-Haz Gown Room	S	8	P	
35	Haz Buffer Room	S	7	P	
36	Haz Buffer Room	S	7	P	
37	Haz Gown Room	S	7	P	
38	Haz Gown Room	S	7	P	
39	Non-Haz Pass-Thru	S	7	P	
40	Non-Haz Pass-Thru	S	7	P	
41	Haz Pass-Thru	S	7	P	

42	Haz Pass-Thru	S	7	P	
43	Ante Room/Office	S	8	P	
44	Ante Room/Office	S	8	P	

Overall, the CURIS HHP™ device proved able to quickly and comprehensively decontaminate an entire mobile cleanroom. With the mobile cleanroom "down for maintenance" and the doors open, the CURIS device and two fans alone achieved biodecontamination of all spaces simultaneously for a sporicidal reduction in less than an hour and a half. Extensive validation via biological indicators, chemical indicators, and air and surface sampling demonstrates consistent efficacy. This study proves the ability to use CURIS' 7% hybrid hydrogen peroxide system within a cleanroom space to achieve a repeatable, validatable, 6-log reduction throughout the space, within equipment, and within the HEPA filtration system.

It should be noted the portable CURIS Core was examined in this experiment; however, additional options are available for separate biodecontamination of individual spaces or equipment via CURIS integration or gaseous treatment of isolated enclosures via CURIS TRINITY™. This would allow the user to continue utilizing certain sections of the mobile cleanroom while fully decontaminating other sections.

Sources

[1]*Cleaning cleanrooms*. Cleanroom Technology. (2010, November 26). Retrieved November 29, 2021, from https://www.cleanroomtechnology.com/news/article_page/Cleaning_cleanrooms/57871.

[2]*Cleanroom classifications.* Cleanroom Classifications (ISO 8, ISO 7, ISO 6, ISO 5). Retrieved April 13, 2022, from https://www.mecart-cleanrooms.com/learning-center/cleanroom-classifications-iso-8-iso-7-iso-6-iso-5/.

[3]Harms, K. (2021, May 24). *Cleaning & Sterilization Tips for the cleanroom*. Terra Universal Blog. Retrieved November 29, 2021, from https://www.terrauniversal.com/blog/cleaning-sterilization-tips-cleanroom/.

[4]Henneman, J. (2022, March). Analysis of Range of Use of a Hybrid Hydrogen Peroxide System for Biosafety Level 3 and Animal Biosafety Level 3 Agriculture Laboratory Decontamination. Retrieved March 10, 2022, from http://doi.org/10.1089/apb.2021.0012.