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ORIGINAL ARTICLE

Analysis of Range and Use of a Hybrid Hydrogen Peroxide System for Biosafety Level 3 and Animal Biosafety Level 3 Agriculture Laboratory Decontamination

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Abstract

Introduction: The applications of fumigation and the challenges that high-containment facilities face in achieving effective large volume decontamination are well understood. The Biosecurity Research Institute at Kansas State University sought to evaluate a novel system within their biosafety level 3 (BSL-3) and animal biosafety level 3 agriculture (ABSL-3Ag) facility.

Methods: The system chosen for this study is the CURIS® Hybrid Hydrogen Peroxide™ (HHP™) system, comprising a mobile 36-pound (16 kg) device delivering a proprietary 7% hydrogen peroxide (H_2O_2) solution. To examine the system's efficacy in multiple laboratory settings, two BSL-3 laboratories (2,281 [65 m^3] and 4,668 ft^3 [132 m^3]) with dropped ceiling interstitial spaces and an ABSL-3Ag necropsy suite (44,212 ft^3 [1,252 m^3]) with 21-foot (6.4 m) ceilings were selected. Biological indicators (BIs) of *Geobacillus stearothermophilus* (1.7×10^6 organisms) on steel spore carriers and H_2O_2 chemical indicators (CIs) were used to provide validation.

Results: After cycle optimization, the smaller laboratory had a total of 60 BIs over two treatments that demonstrated a greater than 6-log reduction of bacterial spores. The larger laboratory (192 BIs) and the necropsy suite (206 BIs) had no BIs positive for spore growth when incubated at 60°C for 24 h per manufacturer's specifications.

Conclusion: Overall successful results through multiple components of this study demonstrate that the HHP device, paired with the pulsed 7% H_2O_2 solution, achieved efficacy regardless of variables in laboratory size and layout. Perceived challenges such as 21-ft (6.4 m) ceiling heights, active equipment, and difficult to access ceiling interstitial spaces proved unfounded. Given the successful sterilization of all challenged BIs, the HHP system presents a useful alternative for high level decontamination within BSL-3 and ABSL-3Ag facilities.

Keywords: fogging, high level disinfectant, sterilant, hydrogen peroxide vapor, decontamination

Background

Biosafety Level 3 (BSL-3) facilities and Animal Biosafety Level 3 Agriculture (ABSL-3Ag) facilities are designed to allow for the study and handling of high-consequence pathogens that affect plants, animals, and food products, including zoonotic pathogens that can infect humans. Guided by Biosafety in Microbiological and Biomedical Laboratories, 6th Edition, principles for biosafety are focused on practices of containment and risk assessment.[1] Decontamination practices reduce potential for cross contamination between research experiments and ensure that new studies start with a pathogen-free space. These laboratories are required to use a reliable and validated process for whole space decontamination.[2]

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It is also of utmost importance that decontamination methods do not harm sensitive and complex laboratory equipment.[2]

Understanding the challenges of various spaces within BSL-3 and ABSL-3Ag facilities is key to achieving proper decontamination and ensuring the microbiological security of the facility. Research performed at BSL-3 and ABSL-3Ag facilities requires the ability to house both plants and livestock of various sizes, while also encompassing the equipment necessary to study microscopic organisms. Among this range of laboratory spaces are interstitial spaces that are normally located adjacent to or above an adjoining laboratory, with varying degrees of separation from the laboratory. One particularly challenging interstitial space is located above the drop ceiling tile grid. The area above the ceiling grid, as well as the laboratory, is subject to contamination risk either from equipment use or procedures that may generate aerosols.

ABSL-3Ag facilities also contain a necropsy laboratory that is designed to facilitate the postmortem examination of infected livestock. Necropsy investigations make these spaces particularly susceptible to exposure to pathogens due to the possible aerosolization and overall presence of bodily fluids. The necropsy suite, due to the nature of the work performed there, is the largest space within a facility. This laboratory can have ceiling heights reaching 21 ft (6.4 m) and contain large laboratory equipment. The equipment present within the laboratory can create additional surface locations where contamination can occur. Effective decontamination of these two unique environments can be difficult to achieve, and as such an ideal decontamination system for these facilities would be able to handle both of these different applications.

The goal for BSL-3 and ABSL-3Ag facilities is to achieve a complete decontamination of research spaces. Indicators containing *Geobacillus stearothermophilus* bacterial spores have become an international standard for validating certain decontamination technologies as it is understood that any decontamination procedure effective against spores is likely to inactivate other biological agents as well.[2] A 6-log reduction of this challenged indicator can provide validation of room sterilization,[3] and for this reason, these biological indicators (BIs) are chosen for validation of new systems as well.

Given the wide range of spaces needing decontamination, it is difficult to find a method that is both highly effective and extremely versatile to serve multiple areas within the facility. BSL-3 and ABSL-3Ag laboratories have historically used a range of decontamination techniques to address contamination concerns such as fumigated formaldehyde, chlorine dioxide gas, or, most recently, vaporized 35–59% hydrogen peroxide (H_2O_2).[2,4] Although these methods are efficacious, they may also be damaging to equipment after many cycles, pose extreme exposure risks for staff, and in some cases require lengthy clean-up of residues left behind.[2,4] Owing to the caustic and hazardous nature of these chemicals, their application must be constantly monitored, which can be both labor intensive and create additional risk for staff.

Introduction

The applications of fumigation as well as the challenges BSL-3 and ABSL-3Ag facilities face in achieving safe, consistent, and effective decontamination are well understood within the industry.[2,4,5] Even as technologies for decontamination advance, the overall goals remain constant. Joslyn describes the ideal fumigant as one that should leave no residues or should be capable of rapid removal to safe levels after fumigation.[5] H_2O_2 disinfection technologies as a whole have demonstrated an efficacy equal to that of traditionally employed methods,[2] paired with advancements in their delivery systems.

The use of H_2O_2 systems in laboratories has been the subject of numerous studies and comparisons.[2,4] Ample research demonstrates the efficacy of high concentration H_2O_2, using a variety of delivery devices. That prior research has highlighted both efficacy and ease of use of equipment as important measurements of a desirable system.[4] As a result, it becomes important to review both use and versatility of a system, relative to those methods that have been commonly applied, when considering its value to a laboratory facility. As H_2O_2-based technologies become increasingly widespread, questions remain as to the applicability and efficacy at varying concentrations. What has not yet been seen is whether or not advancements in lower concentration solutions paired with manufacturing that favors versatility can improve upon historically applied disinfection methods.

With this in mind, this study sought to challenge an advanced design Hybrid Hydrogen Peroxide™ (HHP™) system within the BSL-3 and ABSL-3Ag facilities at the Kansas State University Biosecurity Research Institute (BRI). The HHP system comprises a mobile 36-pound (16 kg) device paired with a 7% solution (CURoxide™). This solution is delivered by the device into the air as a hybrid mixture of vaporous and aerosolized particles. The device functions through pulse technology, which, after the primary injection of HHP into the space, accounts for the natural decomposition of H_2O_2 by periodically replenishing the HHP fog thus enabling an enhanced contact time. Although any fogged chemical requires some degree of attention to safety, this 7% solution falls below the hazardous chemical range of 8% H_2O_2 and well below the industrial use range of solutions >28% H_2O_2 common to traditional systems.[6] In addition, the optimal operating condition of the HHP system is ~139 parts per million (ppm), in contrast to higher concentration systems that operate between 300 and 2,000 ppm.[7]

As a safety measure, the system operates manually with a delayed start or remotely from an electronic tablet. Use of the tablet enables documentation of cycle parameters through the manufacturer software application (CURIS® App). Both control options contain emergency stop capabilities. For validation, commercially available carriers of *G. stearothermophilus* ($\approx 1 \times 10^6$ spores) were used in conformity with the international standard for decontamination by H_2O_2.[2]

Overall, this study sought to determine whether one system could conquer a range of challenges. One specific interest was the ability of the device to deliver a solution capable of migrating around equipment, into adjacent interstitial spaces and attached rooms, as well as the ability to capably treat large open spaces with ceiling heights up to 21 ft (6.4 m). The HHP system was implemented in a manner consistent with the laboratory's existing protocols for gaseous fumigation systems, which includes the use of fans to disperse the fumigant. Although normally not required, the HHP system was operated under similar conditions as these other systems so as not to present additional variables when comparing the different technologies. Within the facility, two particularly challenging areas of the laboratory were chosen for this investigation.

The first study sought to decontaminate two laboratories along with their affiliated ceiling interstitial spaces. In addition to the challenges posed by access around equipment and into small spaces, these laboratories contained working biological safety cabinets (BSCs) that actively filtered the air during treatment. As the HHP system had previously demonstrated efficacy in this application,[8] this further analysis was intended to expand upon that study by adding this challenge to the overall goal of decontaminating the laboratory and its ceiling interstitial space.

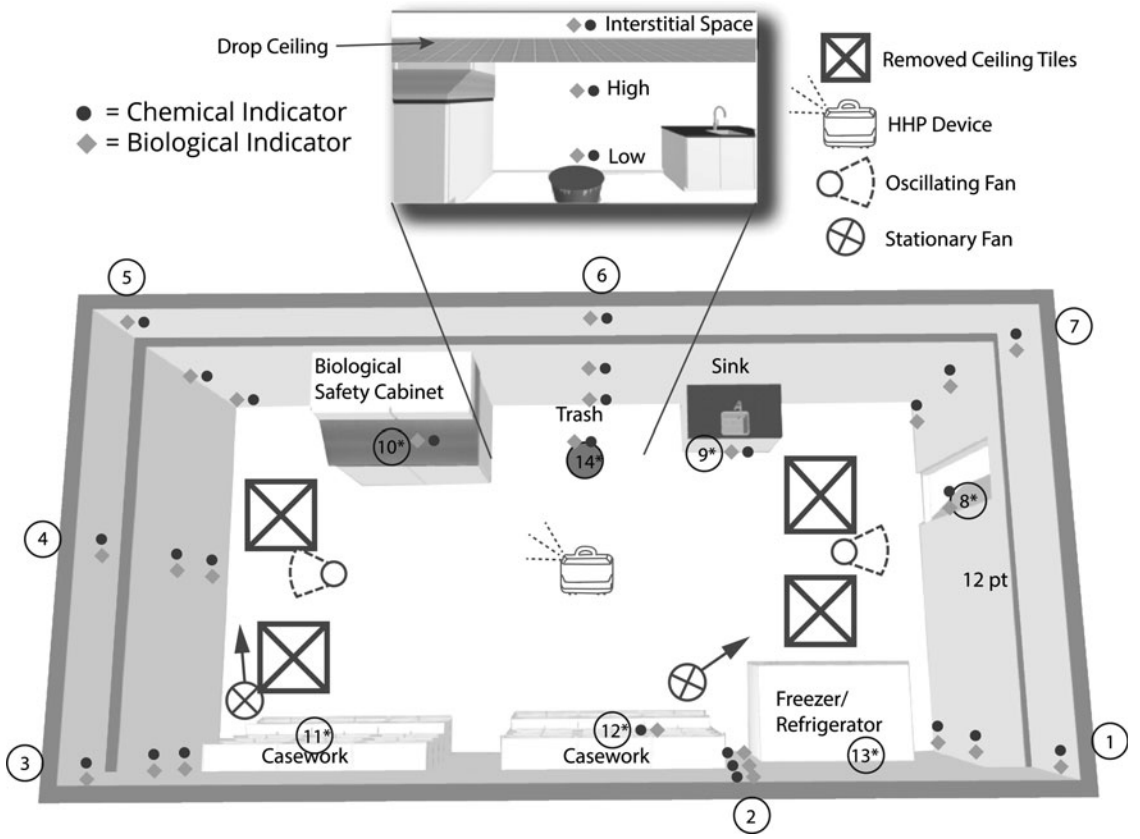

Figure 1. Diagram of Laboratory 1 (2,281 ft^3 [65 m^3]). BIs of *Geobacillus stearothermophilus* (1.7×10^6) and H_2O_2 CIs were placed in pairs throughout each laboratory. Indicator pairs were placed in sets of three: low near the ground, high near the ceiling, and within the interstitial space of the drop ceiling as numbered. Numbers with an asterisk (*) indicate placement of a single indicator pair. Also shown are approximate locations of the removed ceiling tiles, fans, and HHP device. BI, biological indicator; CI, chemical indicator; H_2O_2, hydrogen peroxide; HHP, Hybrid Hydrogen Peroxide.

The second study took place within the necropsy suite, one of the largest spaces within the facility. This study sought to discover whether the small low-profile devices were powerful enough to be used in a space with 21-ft (6.4 m) ceiling heights, walk-in cooler, a soft sided anteroom, two shower block change rooms, and additional equipment. This study also sought to determine the usefulness of this particular technology's ability to pair multiple devices for automated synchronized decontamination. In an effort to establish the range of operation, this study challenged the technology by using one fewer device than the manufacturer-recommended number for the 44,212 ft^3 (1,252 m^3) space.

Collectively, these study components were designed to aggressively test the efficacy and versatility of the HHP system with the intention of discovering the system's ability to match or exceed traditional methods with high-concentration solutions in safety and efficacy to successfully decontaminate laboratories and their contents.

Table 1. Cycle parameters

	Laboratory	Run	Volume (ft^3)	Primary injection (min)	Pulse (min)	BI total
Study 1	1	1	2,281 ft^3 (65 m^3)	16.88	40	60
		2		16.88	40	
	2	1	4,668 ft^3 (132 m^3)	23.5	40	192
		2		23.5	40	
		3		23.5	40	
Study 2	Necropsy	1	44,212 ft^3 (1,252 m^3)	60.9	40	206
		2		60.9	40	

Parameters of the HHP system for each laboratory decontamination cycle. BI, biological indicator; HHP, Hybrid Hydrogen Peroxide.

Materials and Methods

Study 1: BSL-3 Laboratories with Dropped Ceiling Interstitial Spaces

Study 1 tested the HHP disinfection system (CURIS, Oviedo, FL) in two inactive clean BSL-3 laboratories measuring 2,281 (65 m^3) and 4,668 ft^3 (132 m^3). Decontamination runs were performed separately for each laboratory. Both laboratories had standard ceilings and a lower dropped ceiling that consists of ceiling tiles in a metal grid that creates an interstitial space between the two. Environmental conditions ranged from 66.3°F to 75°F (19°C–24°C) and from 20.7% to 52% relative humidity, no conditioning occurred. Doors were sealed and HVAC bioseal dampers were closed during the decontamination process. At least four ceiling tiles were removed in each laboratory to allow for air flow into the interstitial space.

After initial testing and calibration runs, fog and pulse rates were adjusted to ensure H_2O_2 reached all areas of the laboratory. Consistent with routine BRI protocol for gaseous fumigation, at least two oscillating fans were added to the laboratories to create air movement, along with two or more blower fans directed toward the interstitial openings. *G. stearothermophilus* (1.7×10^6 organisms; ATCC 7953; D value at 55°C ± 5°C, 2.3 mg/L 1.0–3.0 min) steel spore carriers, Tyvek®/Tyvek BIs (Crosstex, Rush, NY), were hung in sets of three, each at a different height: one just above the floor, one near the ceiling, and one above the ceiling tile grid in multiple locations around the room. Individual BIs were placed in casework and equipment (Figure 1). Chemical indicator (CI) strips (CURIS) were also placed throughout the room.

One BSC in laboratory 1 and two BSCs in laboratory 2 were on and running for the duration. The HHP device was placed on the floor in laboratory 1 initially, with subsequent runs elevated, and elevated ~36″ in laboratory 2

Table 2. Study 1 biological indicator results

	Heights				
Location no.	Low	High	Interstitial space	Location no.	Results
Laboratory 1 (2,281 [65 m^3])					
1	Pass	Pass	Pass	9*	Pass
2	Pass	Pass	Pass	10*	Pass
3	Pass	Pass	Pass	11*	Pass
4	Pass	Pass	Pass	12*	Pass
5	Pass	Pass	Pass	13*	Pass
6	Pass	Pass	Pass	14*	Pass
7	Pass	Pass	Pass		
8	Pass	Pass	Pass		
Laboratory 2 (4,668 ft^3 [132 m^3])					
1	Pass	Pass	Pass	19*	Pass
2	Pass	Pass	Pass	20*	Pass
3	Pass	Pass	Pass	21*	Pass
4	Pass	Pass	Pass	22*	Pass
5	Pass	Pass	Pass	23*	Pass
6	Pass	Pass	Pass	24*	Pass
7	Pass	Pass	Pass	25*	Pass
8	Pass	Pass	Pass	26*	Pass
9	Pass	Pass	Pass	27*	Pass
10	Pass	Pass	Pass	28*	Pass
11	Pass	Pass	Pass		
12	Pass	Pass	Pass		
13	Pass	Pass	Pass		
14	Pass	Pass	Pass		
15	Pass	Pass	Pass		
16	Pass	Pass	Pass		
17	Pass	Pass	Pass		
18	Pass	Pass	Pass		

BIs of *Geobacillus stearothermophilus* (1.7×10^6) in locations 1–8 (laboratory 1) and 1–18 (laboratory 2) were placed in sets of three: low near the ground, high near the ceiling, and within the interstitial space of the drop ceiling. In locations 9*–14* (laboratory 1) and 19*–28* (laboratory 2), single indicators were placed on and in equipment throughout the laboratory.

for all runs. Room dimensions, including the interstitial area, were preprogrammed into the HHP system's online data management system. Cycle parameters were determined by room volumes and accounted for the operational BSCs (Table 1). The HHP devices were operated remotely on a tablet through the accompanying CURIS App.

After cycle completion and aeration, all indicators were collected. CIs were analyzed for exposure. BIs were transferred into tryptic soy broth with pH indicator (Crosstex). BIs were incubated for 24 h at 60°C (140°F) per manufacturer's specifications and results were recorded (Table 2). Positive BI controls were used accompanying every run to confirm viability of the BIs.

Study 2: ABSL-3Ag Necropsy Suite

Study 2 tested the HHP system in a clean inactive ABSL-3Ag necropsy suite, including a walk-in cooler, and a soft sided anteroom with two connected change rooms, for a total volume of 44,212 ft^3 (1,252 m^3). Four synchronized HHP devices were utilized, each device accounting for 11,053 ft^3. Environmental conditions ranged from 68.7°F (20°C) to 72.9°F (23°C) and from 28.8% to 29.3% relative humidity, no additional conditioning occurred. Exterior pressure-resistant doors and HVAC bioseal dampers were closed. Change room and soft sided anteroom doors were propped open.

BI and CI pairs were hung at three heights: one just above the floor, one midway up the wall, and one near the ceiling in multiple locations around the rooms, including three points on hanging outlet wires in the middle of the room. BIs were also placed on equipment and countertops (Figure 2). Seven fans were placed in the room and four HHP devices were positioned on tables ~36 in (91 cm) from the floor. One fan was an industrial 36 in (91 cm) drum unit, placed in the center of the room pointing upward. Fans, fan placement, and HHP system elevation were included as part of routine BRI protocol. Dimensions for the entire laboratory suite were preprogrammed into the HHP system's online data management system.

The HHP devices were synced and activated remotely through a tablet and the CURIS App. Figure 3 shows the HHP device at startup. Data generated from the HHP devices uploaded to the online data management system,

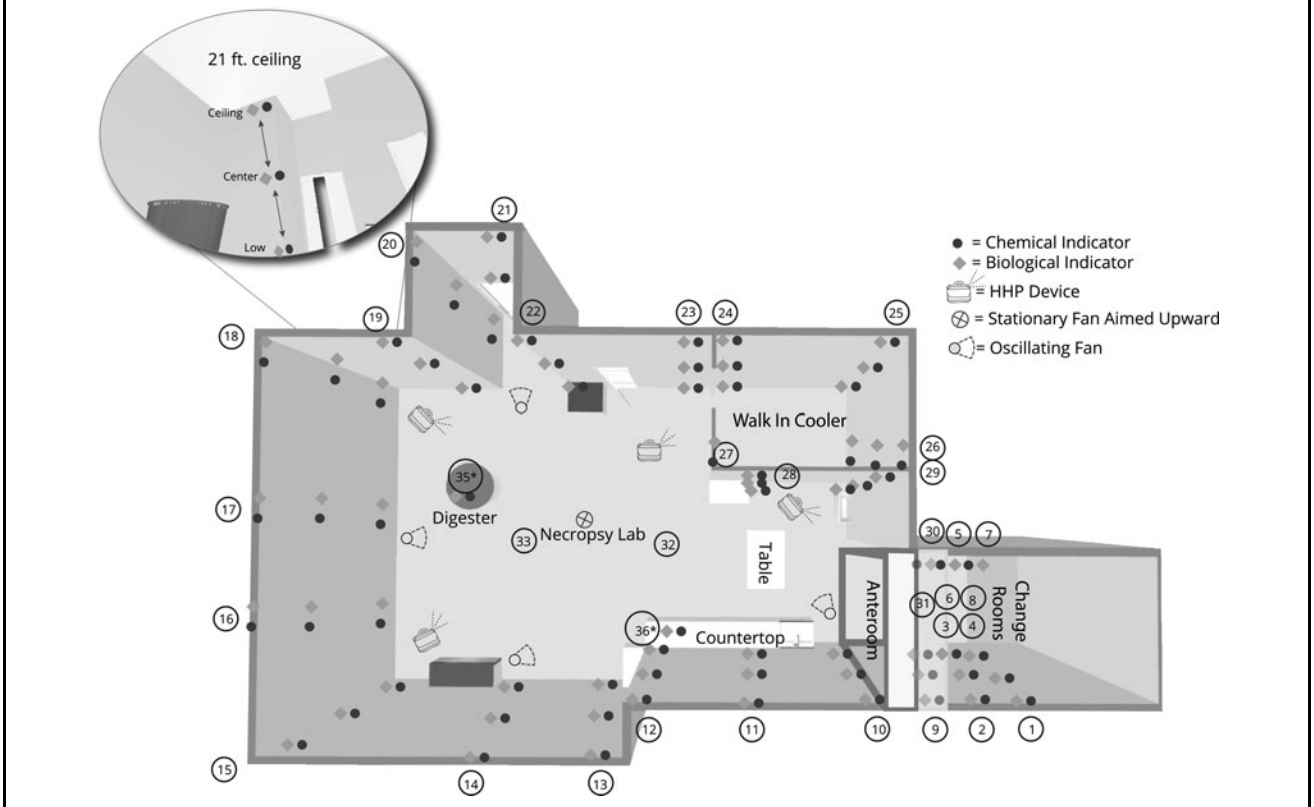

Figure 2. Diagram of the Necropsy Suite (44,212 ft^3 [1,252 m^3]). BIs of *Geobacillus stearothermophilus* (1.7×10^6) and H_2O_2 CIs were placed in pairs throughout the necropsy suite. Indicator pairs were placed at three different heights: low pairs at floor level, center pairs ~10 ft (3 m) high, and ceiling pairs at 21 ft (6.4 m). Single indicator pairs (*) were placed on the digester and on the countertop. Also shown are approximate locations of the fans and HHP device.

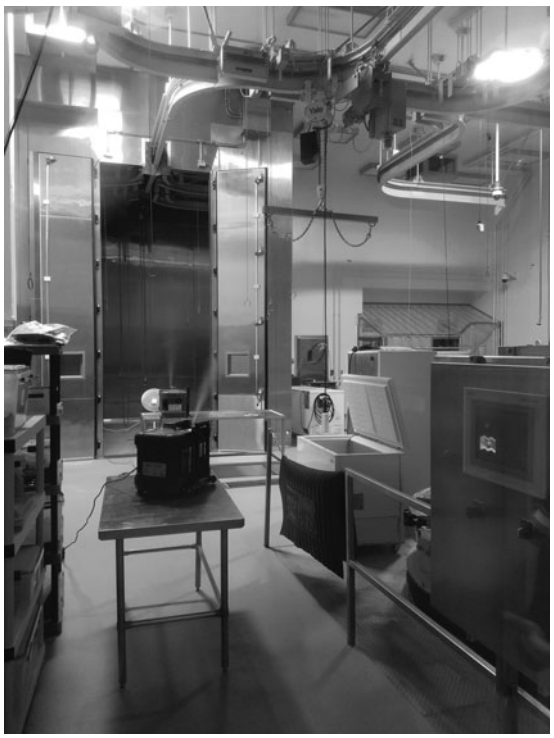

Figure 3. Synchronized HHP devices at primary injection in the necropsy suite.

and treatment reports were generated from that system. After the treatment, bioseal dampers were opened and laboratory doors unsealed for aeration through the building HVAC system. BIs and CIs were collected and processed as in Study 1.

Results

Study 1: BSL-3 Laboratory with Interstitial Space

BIs were analyzed after incubation. All positive controls confirmed viability of the BIs. In the first laboratory, 30 BIs and CIs per treatment were used and demonstrated no bacterial growth for all 30 BIs. Over two treatments, all 60 BIs indicated a >6-log reduction of bacterial spores. CIs were analyzed after cycle aeration, and demonstrated a change in color, verifying the migration of HHP throughout the room (Table 2).

In the second laboratory space, 64 BIs were used per treatment. Over three treatments, no bacterial growth was observed for any of the 192 total BIs, indicating a >6-log sterilization of *G. stearothermophilus* spores was achieved for the entire laboratory, including the ceiling interstitial space (Table 2).

Study 2: ABSL-3Ag Necropsy Suite

All CIs showed a change in color after decontamination, indicating adequate migration of the HHP fog throughout the laboratory space. After incubation, all 103 BIs were negative for bacterial growth, indicating a >6-log sterilization of challenged BIs. Testing was repeated a second time according to the described methods with the same results observed for a combined total of 206 indicators of *G. stearothermophilus* (1.7×10^6) BIs negative for growth (Table 3). All positive controls confirmed viability of the BIs.

Discussion

In decontaminating BSL-3 and ABSL-3Ag facilities, an ideal method is one that is highly efficacious, leaves no residuals behind, and lowers exposure risks for staff. As the internal rooms of laboratory facilities are made up of spaces of all sizes with a variety of equipment, the ideal system would meet these criteria while remaining powerful enough to overcome the inherent challenges. This study was undertaken to determine whether a highly portable HHP device would be capable of addressing the challenges of efficacy and usability while mitigating safety concerns by applying a lower concentration H_2O_2 solution with resulting low ppm.

First, two laboratories measuring 2,281 (65 m^3) and 4,668 ft^3 (132 m^3) with ceiling interstitial spaces including operating BSCs were treated. Testing and calibration runs were performed with minimal displacement of ceiling tiles to establish the decontamination cycle parameters. Fans were used consistent with the laboratory's existing protocol for gaseous fumigation. After this testing, subsequent cycles reported here adjusted primary injection and pulse timing and removed additional ceiling tiles to enhance HHP movement and account for the overhead interstitial space. Full exposure was then achieved by increasing access between the spaces.

As an additional challenge, the BSCs were turned on with the front sash open. As BSCs contain their own high efficiency particulate air filtration systems, this filtration had to be accounted for to not present a variable to disinfection. This was achieved using options within the HHP system's programming that increased the delivered dosage of solution into the space. This study expands on that of Ghidoni et al. that demonstrated simultaneous room and BSC decontamination.[8] The overall success of the BIs points to a successful decontamination and to the capability of the HHP system to achieve decontamination of laboratories and interstitial spaces, as well as within operating BSCs.

The second part of this study treated the 44,212 ft^3 (1,252 m^3) necropsy suite with 21-ft (6.4 m) ceilings, walk-in cooler, soft sided anteroom, and adjacent change rooms. This large space required the use of four synced HHP devices, which operated through the CURIS App. Individually, each device accounted for 11,053 ft^3 (313 m^3). Fans were used to disperse the HHP in a manner consistent with BRI protocols (Figure 2).

Table 3. Necropsy suite biological indicator results

Anteroom							*Necropsy laboratory*						
	Low		*Center*		*Ceiling*			*Low*		*10 ft (3 m) above*		*21 ft (6.4 m) ceiling*	
Location no.	*(BI)*	*(CI)*	*(BI)*	*(CI)*	*(BI)*	*(CI)*	*Location no.*	*(BI)*	*(CI)*	*(BI)*	*(CI)*	*(BI)*	*(CI)*
1	Pass	Pass	Pass	Pass	Pass	Pass	10	Pass	Pass	Pass	Pass	Pass	Pass
2	Pass	Pass	Pass	Pass	Pass	Pass	11	Pass	Pass	Pass	Pass	Pass	Pass
3	Pass	Pass	Pass	Pass	Pass	Pass	12	Pass	Pass	Pass	Pass	Pass	Pass
4	Pass	Pass	Pass	Pass	Pass	Pass	13	Pass	Pass	Pass	Pass	Pass	Pass
5	Pass	Pass	Pass	Pass	Pass	Pass	14	Pass	Pass	Pass	Pass	Pass	Pass
6	Pass	Pass	Pass	Pass	Pass	Pass	15	Pass	Pass	Pass	Pass	Pass	Pass
7	Pass	Pass	Pass	Pass	Pass	Pass	16	Pass	Pass	Pass	Pass	Pass	Pass
8	Pass	Pass	Pass	Pass	Pass	Pass	17	Pass	Pass	Pass	Pass	Pass	Pass
9	Pass	Pass	Pass	Pass	Pass	Pass	18	Pass	Pass	Pass	Pass	Pass	Pass
30	Pass	Pass	Pass	Pass	Pass	Pass	19	Pass	Pass	Pass	Pass	Pass	Pass
31	Pass	Pass	Pass	Pass	Pass	Pass	20	Pass	Pass	Pass	Pass	Pass	Pass
Walk in cooler							21	Pass	Pass	Pass	Pass	Pass	Pass
	Low		*Center*		*Ceiling*		22	Pass	Pass	Pass	Pass	Pass	Pass
Location no.	*(BI)*	*(CI)*	*(BI)*	*(CI)*	*(BI)*	*(CI)*	23	Pass	Pass	Pass	Pass	Pass	Pass
24	Pass	Pass	Pass	Pass	Pass	Pass	28	Pass	Pass	Pass	Pass	Pass	Pass
25	Pass	Pass	Pass	Pass	Pass	Pass	29	Pass	Pass	Pass	Pass	Pass	Pass
26	Pass	Pass	Pass	Pass	Pass	Pass	32	Pass	Pass	Pass	Pass	Pass	Pass
27	Pass	Pass	Pass	Pass	Pass	Pass	33	Pass	Pass	Pass	Pass	Pass	Pass
Other							34	Pass	Pass	Pass	Pass	Pass	Pass
Location no.					*(BI)*	*(CI)*							
35	Placed on digester				Pass	Pass							
36	Placed on countertop				Pass	Pass							

BIs of *Geobacillus stearothermophilus* (1.7×10^6) and H_2O_2 CIs were placed in pairs throughout the necropsy suite. Indicator pairs were placed in sets of three: low near the floor, center at 10 ft (3 m), and ceiling at 21 ft (6.4 m). Pairs are shown beside one another according to the locations within the facility where they were placed.

CI, chemical indicator; H_2O_2, hydrogen peroxide.

For both studies, confirmation of a >6-log reduction was achieved by means of commercially available *G. stearothermophilus* BIs. Over the course of Study 1, all 252 of 252 BIs were negative for growth. Over the course of Study 2, all 206 of 206 BIs tested throughout the necropsy suite were negative for growth. As demonstrated here and in numerous other studies, H_2O_2 is highly efficacious in killing bacterial spores, as well as lower level organisms.[2,8–10] The effective kill of *G. stearothermophilus* spores in BIs placed at the ceiling demonstrates the HHP system was capable of achieving a >6-log reduction even at the challenging height of 21 ft (6.4 m) when coupled with routine BRI protocols.

Along with efficacy, it is equally important to enable ease of use of the decontamination process without introducing risks in the form of chemical exposure. Historically, formaldehyde is used in concentrations ranging from 600 to 1,400 ppm,[4] and vaporized H_2O_2 systems with solution ranges of 35–59% have been shown to operate as high as 2,000 ppm[7]. Although these methods are applied following full safety protocols, there is still an inherent potential danger to an operating ppm that greatly exceeds the occupational safety and health administration permissible exposure limits of 0.1[11] and 1 ppm, respectively.[12] At 7% H_2O_2, the HHP system's lower operating concentration of $\sim$139 ppm[13] may reduce the risks associated with accidental exposure.

Owing to the fortified construction, maintaining connectivity with remote operated devices proved to be challenging in certain areas of the facility. The manual start option is one solution for this, however, creating a job report must then be done through the online data management system. For facilities that must maintain meticulous documentation for all decontamination procedures, the remote start through the CURIS App will be an important operation method if the device is to provide cycle data in an automated reportable format. Should the tablet lose connection during the treatment, connection must be re-established with that device to download the data.

Within the large necropsy suite, the decontamination process was enhanced by the ability of the four synchronized HHP devices to work simultaneously as a network,

delivering the optimal amount of HHP fog needed. This particular design element enhances the versatility of the system when the goal is to achieve a >6-log reduction within large volume laboratory spaces.

Conclusion

This study demonstrated the HHP system's capabilities within the challenging environment of a BSL-3 and ABSL-3Ag facility. Building on these results, it would be of interest to investigate the system's ability to achieve success in more sensitive areas of the laboratory or as part of a response procedure for laboratory spills and accident protocols. As the HHP system has shown efficacy against Norovirus,[10,14] SARS-CoV-2,[9] *Pseudomonas*,[9] *Clostridioides difficile*,[15] and others, as well as some promise in penetrating biofilms and soil loads,[15,16] further study mimicking potential accident conditions would be of interest. Along with the HHP system's effectiveness in BSC decontamination[8] in conjunction with the BSC and ceiling interstitial success presented here, additionally of interest would be decontamination of even more challenging to access areas of the laboratory such as duct work and filter housings.

Overall successful results through multiple components of this study demonstrate that the lower concentration 7% CURoxide solution paired with the portable HHP device achieved efficacy regardless of variables in laboratory size and layout. Perceived challenges such as 21-ft (6.4 m) ceilings, active equipment, and difficult to access ceiling interstitial spaces were unfounded, even when using one fewer device than the manufacturer-recommended number, through incorporation of BRI protocols. As a result, the challenge of decontaminating large volume spaces coupled with high ceilings may not be as insurmountable as previously perceived. In light of the necessity of an efficacious system, without residues and with improved safety for staff, the results of this study demonstrate that decontamination with the HHP system is a viable option for BSL-3 and ABSL-3Ag laboratory decontamination in spaces of all sizes.

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Authors' Contributions

R.R.S., J.D., E.A.M., and A.T. carried out the decontamination setup, cycles, and processing of biological indicators. J.R.H. and R.R.S. conceived, planned, and supervised the decontamination testing. J.R.H. and M.H. wrote the article with support and feedback from all authors to create the final document. All listed authors contributed significantly to this study.

Authors' Disclosure Statement

M.H. is the senior research scientist for CURIS System. All other authors are employees of the Biosecurity Research Institute, Kansas State University.

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