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Biodecontamination of an IVC Rack Using Automated, Integrated HHP Decontamination

Synopsis

This study shows the comprehensive 6-log biodecontamination of an IVC rack within a decontamination chamber using automated, integrated HHP technology, resulting in a repeatable, validatable kill throughout the animal rack and cages.

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Introduction

Industries, including pharmaceutical development and manufacturing, biomedical research, and animal laboratory science, have a common need for animal-based testing. To ensure animal welfare and optimal experiment outcomes, animal housing systems are designed with contamination control, food and water delivery, and ease of use in mind. For example, Individually Ventilated Cages (IVCs) mounted onto a containment rack with a center plenum designed to draw air through the IVCs and out of the rack system. IVCs use low-velocity airflow and filtration, which helps prevent cross-contamination between cages by minimizing the spread of airborne particles. Further, the single-directional airflow improves air quality by lowering humidity and removing waste gases, such as carbon dioxide (CO_2) and Ammonia (NH_3) (Figure 1).

Figure 1. IVC rack with cages. Pictured is the circular IVC rack with washed IVCs in the chamber prior to decontamination.

Traditional IVC Rack Decontamination

Following animal research guidelines, IVCs, IVC racks, and associated equipment must be washed, cleaned, and sterilized prior to occupation and at ≤ 14-day intervals while in use.[1] Common current sterilization practices include autoclave steam sterilization and dry heat sterilization. While effective, these methods can be time-consuming and detrimental to the polycarbonate material of the IVCs, decreasing the lifespan of this costly equipment.[2] Additionally, several modern methods employ high-concentration chemical systems, such as 35% vapor phase hydrogen peroxide (VPHP), which requires enhanced safety practices and could pose a risk to staff.[3]

Ancillary Costs of IVC Rack Decontamination

For animal research facilities, decreasing costs by increasing the longevity of specialized primary enclosures such as IVCs could significantly reduce operating expenses. Equally, methods such as steam sterilization come with a high utility cost, whereas more modern systems may deliver efficacious sterilization outcomes without the enormous use of natural resources, such as water, steam, and electricity. Potential cost savings, ease of practices, and sustainability have all led facilities to look for better decontamination methods for their animal housing systems.

Exploring Alternatives—HHP Technology
Finding efficacy failures with previously employed legacy methods of 35% VPHP and 8% ionizing peroxide systems, the University of Houston Animal Resource Center approached CURIS System to study the feasibility of using CURIS technology for the decontamination of their IVC racks and IVCs.

CURIS System offers a range of custom products with integration technology for use in specified laboratory areas or within laboratory equipment such as isolators and chambers. This integration technology uses CURoxide, a proprietary 7% hydrogen peroxide that is delivered as Hybrid Hydrogen Peroxide (HHP), a mixture of vaporous and gaseous particles. CURIS' HHP technology is used for decontamination in multiple laboratory settings, including biological safety (BSL),[4] pharmaceutical research, and manufacturing facilities; decontaminating biocontainment laboratory equipment;[5,6] and contamination control in human clinical settings.[7] The established use of this technology for high-level decontamination made it a natural choice for animal housing systems.

To investigate this application of CURIS HHP technology for IVC rack decontamination, a feasibility study was undertaken by the University of Houston to determine if using HHP could achieve desired decontamination goals sustainably where others have failed without the waste of utilities and materials.

Materials

CURIS 7000fi (CURIS System; Oviedo, FL)
IVC cages (Animal Care Systems; Centennial, CO)
IVC Optimice Rack, Rack-Mounted Avidity/Edstrom Valves C776O2EDR (Animal Care Systems; Centennial, CO)
IVC rack blower model AFB0812EHE (Animal Care Systems; Centennial, CO)
Lynx decontamination chamber (Lynx Product Group; Wilson, NY)
Biological Indicators (True Indicating; Toledo, OH)
Chemical Indicators (3M; St. Paul, MN) (Black & White CIs)
Hygrometer (Amprobe; Everett, WA)
H2O2 meter (ATI; Novi, MI)

Methods

Testing took place in the University of Houston's Animal Resource Center inside a stainless-steel door decontamination chamber fitted with CURIS 7000fi technology. The 7000fi system consists of integrated technology with components that control humidity, air movement, HHP injection, and other parameters through external touchscreen controls.

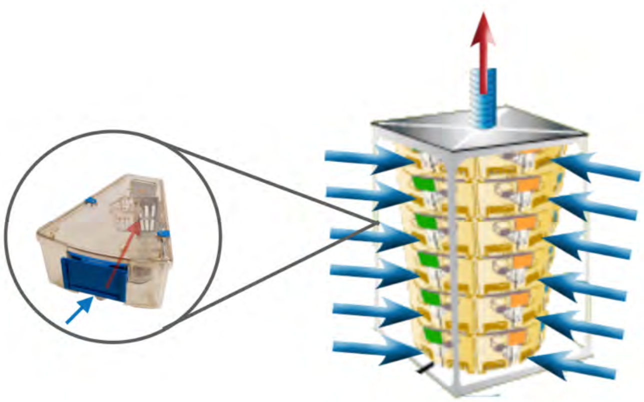

Figure 2. Circulation pathway.
This image shows the airflow through IVCs into the IVC Rack plenum and circulated out the attached blower. This same circulation system was used during the HHP cycle for decontamination of the IVCs, IVC rack, inner plenums, and attached blower. *Adapted from AnimalCareSystems.com*[8]

The circular IVC rack system with washed IVCs was placed into the decontamination chamber. The IVCs were designed to fit on the circular rack with the filtered air inlet on the outer lower front side of the cage. In addition, this particular IVC rack system was fitted with an exhaust blower designed to draw air through the cages and out the top of the rack system. During the decontamination cycle, the air drawn through the blower cycled back into the chamber (Figure 2).

To determine the efficacy of the decontamination process, chemical indicators (CIs) were used on the rack outside the IVCs. Biological indicators (BIs) and CIs were placed within enclosed individual cages with Reemay® 2024 spunbonded polyester filter media in place (Figure 3). Chemical indicators are color-based, changing color to indicate exposure to hydrogen peroxide. The BIs used for this study contained *Geobacillus stearothermophilus* (1 x 10^6 spores) encased in Tyvek/Tyvek pouches, a standard for monitoring hydrogen peroxide decontamination.[9] Two types of CIs were placed inside the cages with each BI to visualize the distribution of HHP (Figures 4a and 4b).

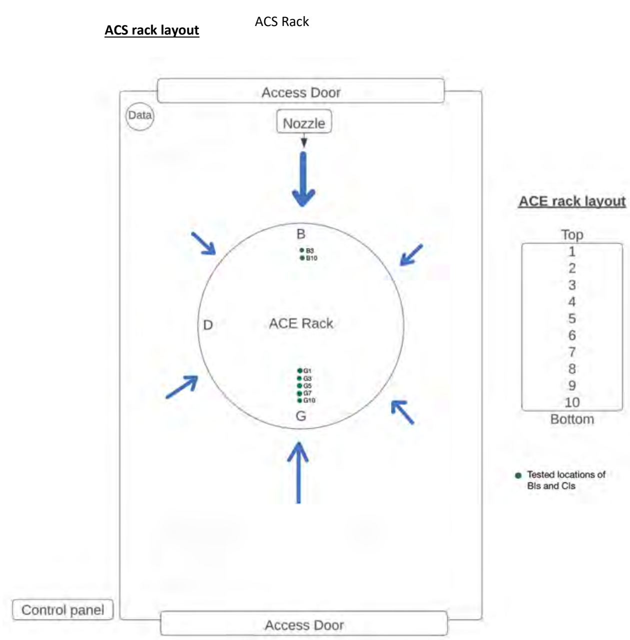

Figure 3. Diagram of tested locations.
Layout of the chamber with locations of the IVC rack and integration components.

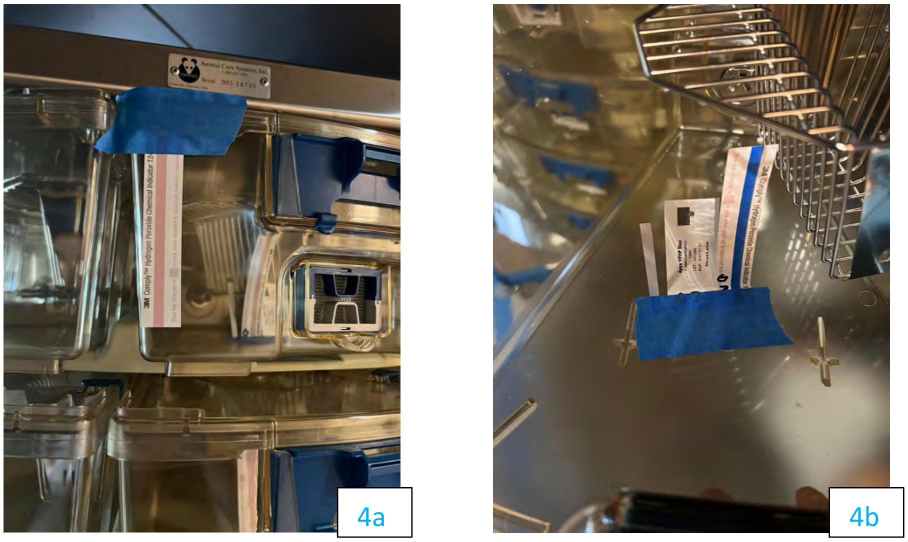

Figure 4a and 4b. Indicator placement.
Images of chemical indicators outside the IVCs (4a) and biological and chemical indicators placed inside individual cages (4b).

The chamber was closed and conditioned for decontamination. The automated HHP cycle was initiated remotely via the touchscreen controls. Following the HHP cycle, normalization was initiated, returning the chamber to safe levels for re-entry.[10] Upon re-entry, BIs were aseptically processed into growth media, incubated at 56° C, and monitored for seven days. Results were recorded. In addition, CIs were collected and photographically recorded on-site.

Results

The testing cycle was completed in ≤3 hours from injection to safe levels for re-entry.[10] No material incompatibilities of any kind were observed. Following testing and incubation, all BIs demonstrated a sporicidal 6-log reduction on challenged indicators inside the cages (Figure 5a). A positive (unexposed to decontamination) control BI verified the integrity of the indicators. Both chemical indicators placed inside cages and outside the IVC rack demonstrated a thoroughly dispersed exposure to hydrogen peroxide fog (Figure 5b).

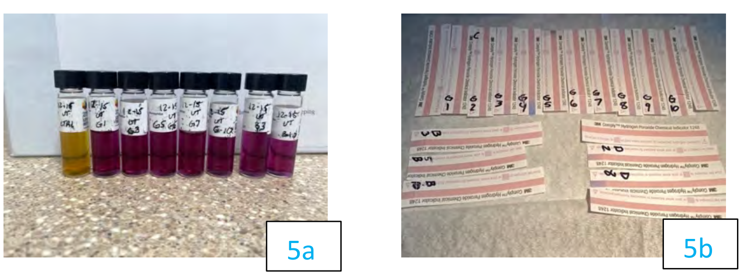

Figure 5a and 5b. Indicator results.
Biological indicator (5a) and chemical indicator (5b) results following the hybrid hydrogen peroxide (HHP) cycle. Biological indicators show 6-log sporicidal decontamination in their purple color indicating no growth present, with the positive control in yellow confirming the integrity of the indicators. Chemical indicators show a pink color confirming the even distribution of HHP throughout the chamber and in individual IVCs.

Discussion

When determining the feasibility of using a new technology, there are multiple areas of concern for facilities. In addition to validated efficacy, feasibility also entails the overall costs of the system, including budgets, staffing, downtimes, utility requirements, and the environment.

Efficacy

Indicator results demonstrated complete decontamination outside the IVC rack and inside individual IVCs on multiple rows and varying heights. This result is particularly notable due to the filtration media on each IVC, as filters can present a barrier to effective decontamination for some vaporous technologies. Here, HHP demonstrated no challenges penetrating filtering materials, proven by the 6-log sporicidal efficacy validated by the BIs and CIs within tested IVCs. Further, while some IVC rack blower systems ventilate into a facility's exhaust system, the tested blower in this study was contained within the test chamber. Since the blower circulated the HHP throughout the chamber environment, this process decontaminated the IVCs, rack, and blower system alike.

Expenses

Operation expenses remain a top interest for vivarium facilities. It is well known that traditional methods of decontaminating IVCs can be detrimental, leading to cloudy and brittle materials that require replacement. It is therefore desirable that sterilizing methods are effective and more compatible with materials, leading to the sustainability of this costly equipment. While material compatibility of IVCs was not the specific focus of this feasibility study, previous work has determined that the lower concentration HHP demonstrates more excellent material compatibility than some traditional legacy methods.[11,12] This demonstrated material compatibility indicates that replacing traditional sterilization methods with HHP could increase the longevity of IVC use, aiding in both sustainability and budgetary goals.

Cycle Time

This feasibility study tested HHP cycle parameters at ≤3 hours from the start of the HHP cycle to safe levels for reentering the chamber. While overall time was not a focus of this study, the clearly demonstrated efficacy of this testing signals that this timeframe would likely be reduced in an optimized setting. For facilities with time-sensitive operations, the potential for a quick turnaround time maximizes the inherent benefits of time savings. Additionally, peak operating concentration levels for the HHP system of 80-150 parts per million, fall well below known levels of operation for similar VPHP technology.[4,13] These lower levels of operation contribute to decreased cycle times and increased safety margins for staff.

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Sustainability

Many long-standing facilities operate with large autoclave equipment dating to facility construction. While age alone does not necessarily make equipment obsolete, methods allowing more sustainable use of utilities have improved over time. Newer systems such as the CURIS 7000fi can bring large cost savings and greater sustainability by lowering overhead utility costs (Figure 6). For new facilities, the smaller integration system eliminates costs in structural building design and infrastructure, as it does not require large, dedicated spaces that older equipment needs.

Beyond cost, many older systems have become outdated, lacking manufacturer support and regular updates. As with this study, performed on-site at the University of Houston, 7000fi technology is custom installed and supported by CURIS System, creating a seamless and supported integration for facilities.

Figure 6. Sustainability Initiatives and Potential Savings.
Areas of potential increased sustainability and decreased cost for vivarium facilities when comparing an HHP system integration to a traditional steam sterilizer include:

- Reduced electrical consumption
- Reduced water use
- No liquid waste and affiliated sewage costs
- No radiation of heat, lowering the need for cooling system operation
- Reduced need for material handling equipment in decontaminating cages and rack as a single unit
- Reduced infrastructure needs
- Lowered preventative maintenance costs and manufacturer support
- Potential increase in longevity for IVCs through greater material compatibility

Conclusion

In conclusion, this feasibility study proves that the 7000fi HHP system, commonly used for laboratory and equipment decontamination processes, is also efficacious for IVC rack systems. The results of this study present laboratories with an alternative to traditional methods in low-consequence, low-concentration HHP technology, offering ease of use, material compatibility, and efficacy with the potentials for increased sustainability and lowered vivarium costs.

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