

Article

Evaluation of a Novel Hydrogen Peroxide Vaporization Robot System for Biodecontamination Compared with Conventional Methods [†]

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Abstract

Hydrogen peroxide vaporization (HPV) is an established no-touch environmental disinfection technology; however, conventional systems require manual setup and may involve prolonged operating times. This study evaluated a newly developed HPV Smart Robot designed to automate hydrogen peroxide vapor distribution and aeration in a controlled clinical simulation environment. A comparative performance evaluation was conducted in a 48.7 m² clinical simulation enclosure. Four experimental cycles were performed, including one conventional HPV cycle and three robotic HPV cycles operated under different navigation modes and fan-angle configurations. *Geobacillus stearothermophilus* biological indicators (BIs) were used to assess sporicidal efficacy, while chemical indicators and hydrogen peroxide concentration measurements were used to evaluate vapor distribution. Complete biodecontamination was achieved at all 20 environmental sampling locations in every experiment. When four robot-internal sampling sites were included, biodecontamination rates ranged from 95% to 100%, with a single persistent positive biological indicator detected at a recessed fan–corner junction within the robotic platform. All experiments achieved hydrogen peroxide concentrations above the manufacturer-recommended target threshold (≥ 140 ppm). Although the robotic system demonstrated greater spatial variability in hydrogen peroxide concentration than the conventional system, effective sporicidal activity was maintained throughout the enclosure. The robotic platform reduced setup time from approximately 15 min to 5 min and shortened total cycle duration from approximately 285 min to 221–231 min. In this controlled simulation study, the HPV Smart Robot demonstrated high microbiological efficacy and improved operational efficiency compared with a conventional HPV workflow. These findings support the feasibility of robotic HPV biodecontamination systems while highlight-

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ing the importance of airflow optimization and internal component design to ensure consistent vapor penetration.

Keywords: hydrogen peroxide vaporization; robotic disinfection; biodecontamination; infection prevention and control; healthcare-associated infections; environmental decontamination

1. Introduction

The COVID-19 pandemic highlighted major challenges in infection prevention and control (IPC), including shortages of healthcare personnel and increased demand for environmental cleaning and disinfection [1–4]. During periods of high patient volume, healthcare workers were frequently required to perform additional environmental decontamination tasks beyond their routine clinical responsibilities, increasing workload and potential occupational exposure risks [5–8]. These challenges emphasized the need for automated and efficient environmental disinfection technologies.

Hydrogen peroxide vaporization (HPV) is a well-established no-touch room disinfection technology with proven sporicidal activity and broad-spectrum antimicrobial efficacy against bacterial spores, multidrug-resistant organisms, fungi, and enveloped viruses, including SARS-CoV-2 [9–12]. Previous studies have reported microbiological reductions exceeding 6-log₁₀ for resistant bacterial spores and near-complete elimination of healthcare-associated pathogens following HPV room decontamination [13,14]. Compared with ultraviolet (UV)-based systems, HPV provides superior penetration into shadowed areas and more reliable inactivation of resistant microorganisms, making it particularly suitable for high-level environmental biodecontamination [13,14].

Despite these advantages, conventional HPV systems typically require manual placement of vapor generators, circulation devices, and scrubbers, and their performance may be influenced by room geometry and airflow patterns. Because circulation devices remain fixed during operation, vapor distribution may become uneven in complex environments [15–17].

To address these limitations, robotic HPV platforms have recently been introduced to automate device positioning, vapor circulation, and environmental monitoring [16,18–20]. While most published studies have focused on operational feasibility or environmental decontamination outcomes, direct microbiological comparisons between robotic HPV systems and conventional HPV technologies remain limited. Consequently, benchmarks regarding sporicidal efficacy, vapor distribution, and operational efficiency of robotic HPV systems have not been fully established.

Preliminary findings from this study were previously presented as a conference abstract at IDWeek 2025 [21]. The present full-length article expands upon that preliminary report by providing a detailed description of the robotic platform and experimental procedures, site-specific biological indicator results, hydrogen peroxide concentration variability analyses, operational time metrics, and an expanded assessment of safety, regulatory considerations, and study limitations.

Therefore, the primary objective of this study was to conduct a preliminary comparative performance evaluation of a newly developed HPV Smart Robot and a conventional HPV system in a controlled clinical simulation enclosure. Specifically, we compared sporicidal efficacy, hydrogen peroxide vapor distribution, and operational cycle time to provide foundational data regarding the performance characteristics of robotic HPV biodecontamination systems.

2. Materials and Methods

2.1. Study Design and Setting

This study was conducted from 2 to 4 April 2024 in the Infectious Disease Clinical Research Simulation Enclosure at Soonchunhyang University Hospital, Cheonan. The enclosure covers an area of 48.7 m² and was designed to simulate a clinical environment for environmental biodecontamination testing.

Temperature and relative humidity were continuously monitored using calibrated sensors before HPV initiation. Environmental conditions ranged from 17–22 °C and 32–50% relative humidity (RH), values that are known to influence hydrogen peroxide vapor distribution, condensation behavior, and biodecontamination efficacy. All experiments were conducted within environmental ranges commonly recommended for HPV applications and reported in previous studies [14,15,22,23]. Environmental conditions for each experimental cycle are summarized in Table 1.

Table 1. Experimental Conditions.

Conditions	1st Experiments (Traditional Methods)	2nd Experiments	3rd Experiments	4th Experiments
System location	Lab Central	Lab wall	Lab wall	Lab wall
Temperature and humidity	17 °C to 18 °C 32% to 40%	17 °C to 18 °C 47% to 50%	21 °C to 22 °C 42% to 47%	21 °C to 22 °C 45% to 47%
Robot travel paths	X	Direct manipulation	Apply Magnetic Lines	Apply Magnetic Lines
Injection time *	2 h	1 h 40 min	1 h 40 min	1 h 30 min
Dwell time	30 min	30 min	30 min	30 min
Aeration time	2 h	1 h 40 min	1 h 40 min	1 h 30 min
Angle of the FAN	Fix the circulator in multiple directions (4 total)	Spread 45 degrees Aeration 90 degrees	Spread 45 degrees Aeration 0 degrees	Spread 90 degrees Aeration 0 degrees

Note 1: Environmental conditions measured immediately prior to each cycle; monitored continuously during exposure and aeration phases. * Injection times were adjusted (2 h for conventional; 1 h 30–40 min for robotic trials) according to baseline humidity and real-time vapor concentration monitoring. The shorter injection periods used in robotic experiments were determined based on preliminary performance testing demonstrating that the integrated circulation fans achieved the target hydrogen peroxide concentration (≥ 140 ppm) more rapidly than the conventional system while maintaining adequate vapor distribution. Note 2: Temperature and humidity values represent the observed ranges recorded throughout each experimental cycle. “X” indicates that no robotic navigation path was applicable because the conventional stationary HPV system was used.

The study protocol was approved by the Institutional Review Board of Soonchunhyang University Cheonan Hospital (IRB No. 2023-05-041-007), and the requirement for informed consent was waived in accordance with the Declaration of Helsinki.

2.2. Biological Indicators and Inoculation

Standard *Geobacillus stearothermophilus* ATCC 7953 biological indicator carriers (model SP-1410; Mesa Laboratories, Inc., Bozeman, MT, USA), certified according to ISO 11138-1/3 and supplied with a manufacturer-specified D-value of 1.5 min at 132 °C, were used as challenge organisms [9,14,15]. These spores were selected because of their high intrinsic resistance to oxidizing agents and their widespread acceptance as conservative indicators for validating hydrogen peroxide-based biodecontamination.

Each test surface was inoculated in triplicate with 0.1 mL of a standardized *G. stearothermophilus* suspension (10^7 CFU/0.1 mL) and air-dried for 2 h before HPV exposure. A total of 24 inoculation sites representing healthcare-relevant materials, including stainless steel, plastic, fabric, leatherette, and rubber, were evaluated during each experimental cycle (Figure 1). ISO 11140-1:2014-compliant hydrogen peroxide chemical indicators (Class

1; Mesa Laboratories, Inc., Bozeman, MT, USA) were co-located with biological indicators to assess vapor distribution [24].

Twenty inoculation sites were distributed throughout the enclosure and used to evaluate environmental biodecontamination efficacy. Four additional inoculation sites were positioned within the robotic platform to assess hydrogen peroxide vapor penetration into enclosed robot-internal structures. Because the conventional HPV system did not include a robotic platform, only the 20 enclosure sites were used for direct comparisons across all experimental conditions, whereas the robot-internal sites were evaluated separately during robotic experiments.

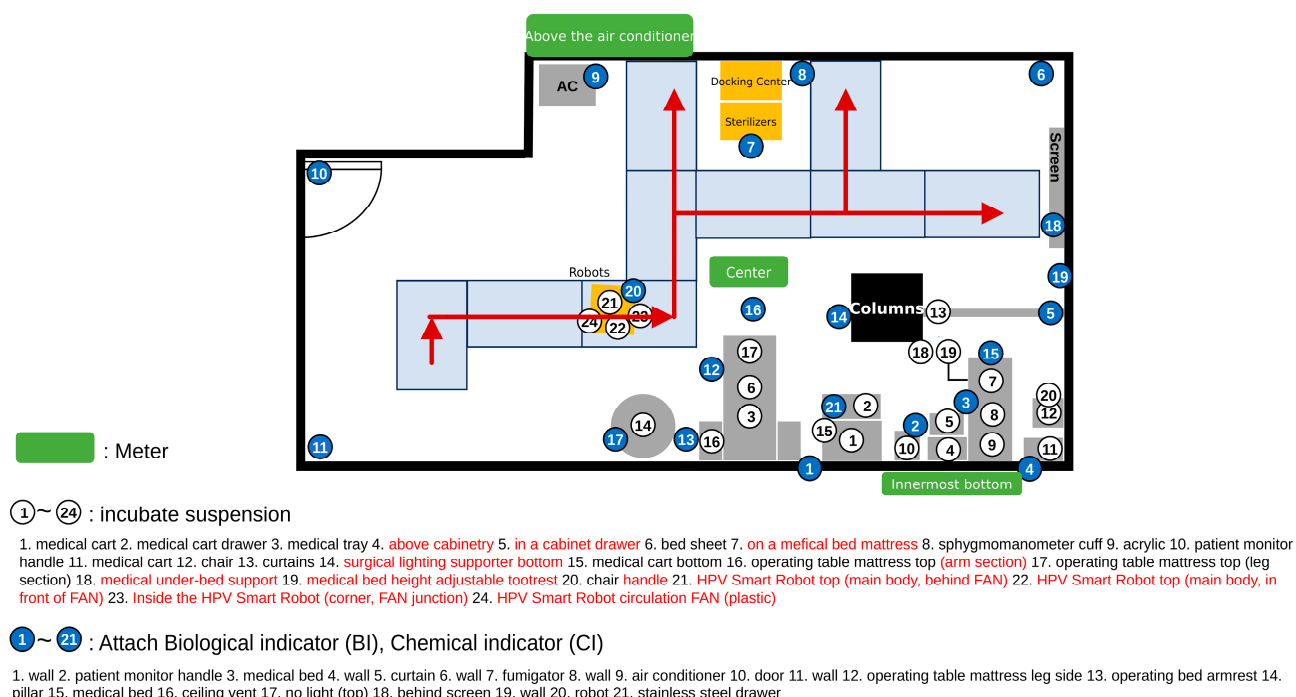


Figure 1. Layout of the infectious disease clinical simulation enclosure showing the locations of biological indicators (BIs), chemical indicators and hydrogen peroxide concentration meters (green rectangles). During robotic experiments, the HPV Smart Robot operated as a mobile platform and followed predefined travel paths within the enclosure. Arrows indicate the direction of robot movement used during the automated navigation experiments. Twenty inoculation sites were located throughout the enclosure, while four additional inoculation sites were positioned within the robotic platform for internal decontamination assessment. Sites 7 and 21 represent locations where incomplete chemical indicator color change was observed.

2.3. HPV Systems

The HPV Smart Robot (Hydrogen Peroxide Vaporization Smart Robot system; Figure 2) is a mobile robotic platform (650 × 650 × 700 mm, W × L × H) designed to automate hydrogen peroxide vapor distribution and aeration during environmental biodecontamination. The system consists of two integrated components: (1) a portable hydrogen peroxide vaporization unit responsible for hydrogen peroxide vapor generation and delivery, and (2) a mobile robotic platform equipped with circulation fans, scrubber modules, navigation sensors, and environmental monitoring sensors.

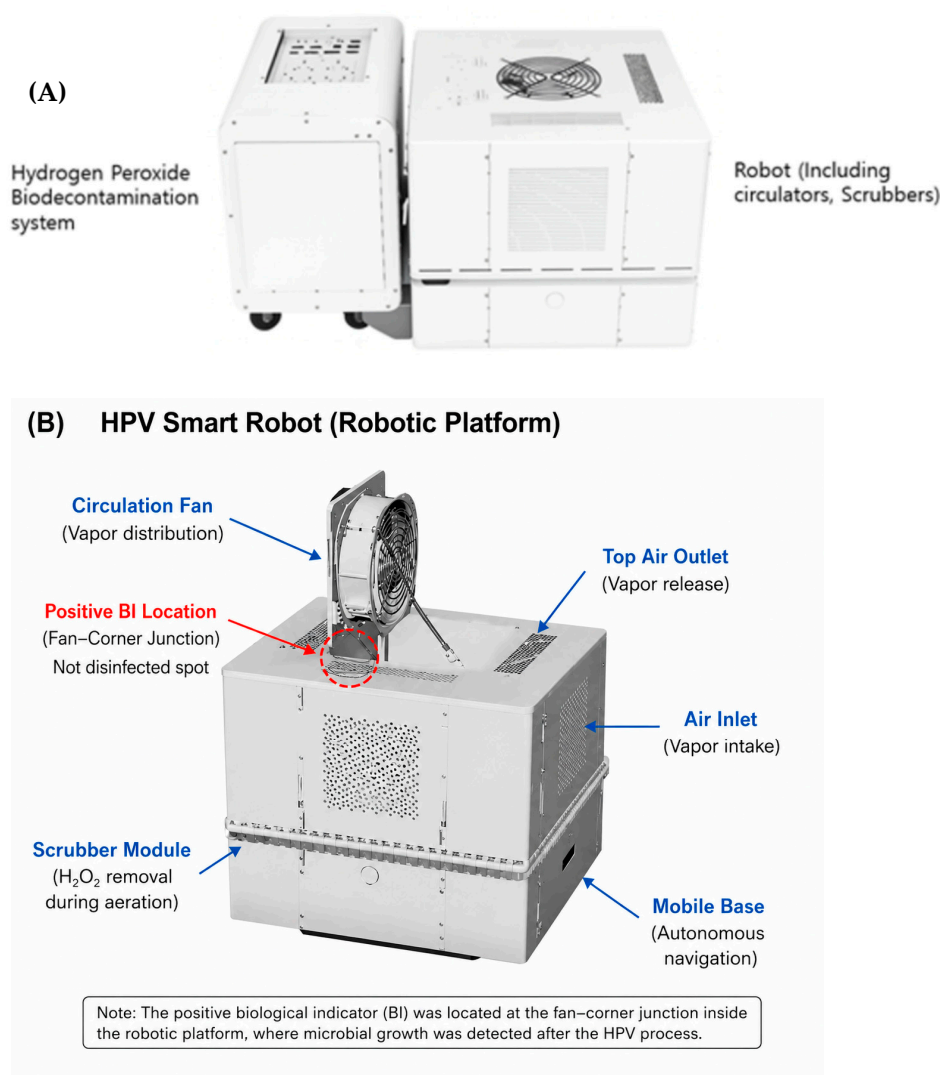


Figure 2. (A) Components of the HPV Smart Robot system. The system consists of a portable hydrogen peroxide vaporization unit (IVH-ER), a mobile robotic platform equipped with integrated circulation fans and scrubber modules, and navigation sensors for autonomous movement. The vaporization unit generates and delivers hydrogen peroxide vapor, while the robotic platform enhances vapor circulation, performs aeration through onboard scrubbers, and supports automated operation within the disinfection environment. (B) Annotated robotic platform of the HPV Smart Robot system. The figure illustrates the major functional components involved in vapor circulation and aeration, including the circulation fan, air inlet, air outlet, scrubber module, and mobile robotic base. The red circle indicates the fan–corner junction, which was the only location within the robotic platform where a biological indicator remained positive after HPV treatment.

The robotic platform incorporates a vertical circulation fan (250 mm diameter, 1120 CFM, 2750 rpm, 140 Pa; W1G250-HJ14-02, ebm-papst Mulfingen GmbH & Co. KG, Mulfingen, Germany) and onboard sensors for continuous monitoring of hydrogen peroxide concentration, temperature, and relative humidity. Navigation sensors enable autonomous movement along predefined travel paths, while adjustable fan-angle configurations facilitate hydrogen peroxide vapor distribution throughout the enclosure and improve environmental coverage.

Hydrogen peroxide vapor generated by the vaporization unit is circulated by the robotic platform, and real-time sensor feedback is used to monitor environmental conditions and confirm attainment of the target concentration threshold (≥ 140 ppm). Residual hydro-

gen peroxide vapor is subsequently removed through integrated scrubber modules until concentrations decrease to safe re-entry levels (<1 ppm). All operational functions, including navigation, vapor distribution, environmental monitoring, and aeration control, are managed autonomously through onboard control software.

For comparison, a conventional heated vaporization system (Huen IVH ER, Huons Meditech® Co., Ltd., Seongnam, Republic of Korea) was used. This system generates hydrogen peroxide vapor through controlled heating and incorporates “Air Cushion” technology to enhance vapor dispersion. The device is certified with Korea KC, European CE, and Russia EAC markings.

2.4. Experimental Cycles and HPV Parameters

Both systems were evaluated across four independent experimental cycles. The first cycle employed the conventional Huons® system with stationary circulators and scrubbers positioned centrally within the enclosure. The second to fourth cycles were conducted using the HPV Smart Robot under different navigation modes and fan-angle configurations. The robot was manually guided during the second cycle and operated using automated magnetic-line navigation during the third and fourth cycles.

Prior to the comparative experiments, preliminary performance testing was performed to evaluate hydrogen peroxide vapor generation, spatial distribution, and attainment of the target hydrogen peroxide concentration. These evaluations demonstrated that the HPV Smart Robot consistently achieved the manufacturer-recommended target concentration threshold (≥ 140 ppm) within a shorter period than the conventional HPV system. Accordingly, injection durations of 90–100 min were selected for the robotic experiments to maintain effective vapor exposure while reducing total cycle time and minimizing excessive condensation. Hydrogen peroxide concentrations were measured using concentration meters positioned within the enclosure, as illustrated in Figure 1.

For concentration variability analysis, mean concentrations, standard deviations (SD), and 95% confidence intervals were calculated using measurements obtained during the plateau phase after attainment of the target concentration threshold. The ≥ 140 ppm threshold was based on manufacturer-recommended operating conditions and previously reported vaporized hydrogen peroxide concentrations used for environmental biodecontamination [22,23]. Effective biodecontamination was achieved through both attainment of the target concentration and maintenance of a subsequent 30-min dwell phase.

The injection phase refers to active hydrogen peroxide vapor generation and dispersion into the enclosure. The dwell phase represents the period during which the target concentration is maintained to maximize microbial inactivation. During the aeration phase, residual hydrogen peroxide vapor is decomposed and removed through the scrubber system until concentrations decrease to safe re-entry levels (<1 ppm).

Each experimental cycle represented a single independent operational evaluation performed under predefined environmental and operating conditions. The multiple inoculation sites within each cycle were used as technical sampling locations to assess spatial biodecontamination performance and vapor distribution and should not be interpreted as independent experimental replicates.

2.5. Safety and Equipment Compatibility

HPV procedures were conducted in unoccupied rooms, and medical devices were either removed or shielded in accordance with manufacturer safety guidance and hospital infection control protocols [14,22]. Post-disinfection inspections were performed to verify the absence of visible surface damage or functional impairment. This approach aligns with regulatory and industry technical guidance, including U.S. Environmental Protection Agency (EPA) assessments and manufacturer documentation describing the compatibil-

ity of non-condensing hydrogen peroxide vapor with sensitive equipment [9,25]. Medical and monitoring devices remained in the room to reflect clinical conditions, and post-cycle evaluations confirmed no abnormalities [14,23].

2.6. Microbiological Recovery and Outcome Measures

Following HPV exposure, the entire inoculated area (approximately 25 cm² per test site) was sampled using polyester-tipped flocked swabs (RegenBio Co., Ltd., Gimpo, Republic of Korea) moistened with sterile distilled water. The same predefined sampling area was used for all experimental sites to ensure methodological consistency across experiments. Samples were immersed in peroxide-quenching Tryptic Soy Broth (TSB-BP16; Mesa Laboratories, USA) and incubated at 55–60 °C for 48 h, with extended incubation to 7 days in accordance with ISO 11138 to detect sublethally injured spores [26]. Complete inactivation was defined as no detectable CFUs. Because the detection limit was 1 CFU per site, complete inactivation corresponded to $\geq 7\text{-log}_{10}$ reduction.

Two control groups were included: a negative control (uncovered TSB exposed to air) and a positive control (TSB inoculated with the spore suspension only). All procedures were performed by three researchers under the supervision of an infection control expert with over 15 years of experience.

The primary outcome was the number of *G. stearothermophilus* recovered after HPV exposure. Secondary outcomes included hydrogen peroxide vapor distribution and aeration characteristics. Vapor uniformity was assessed using co-located chemical indicators and serial concentration measurements at multiple locations (Figure 1). Hydrogen peroxide clearance to safe levels was determined by serial monitoring during aeration.

2.7. Statistical Analysis

Hydrogen peroxide concentration data were summarized descriptively using mean values, standard deviations, and 95% confidence intervals. Because concentration measurements represented repeated spatial observations obtained within the same experimental cycle, formal inferential statistical comparisons between experimental conditions were not performed. Biodecontamination rates were reported descriptively due to near-complete inactivation precluding inferential statistical analysis ($p < 0.05$).

2.8. Regulatory Context

This study was designed as a controlled comparative performance evaluation rather than as a regulatory conformity or certification study. Therefore, it was not conducted in accordance with formal room disinfection certification standards such as EN 17272 [27] or AFNOR T72-281 [28], which specify requirements for type-testing of airborne room disinfection systems. Biological indicator samples were collected only after aeration when residual hydrogen peroxide concentrations had decreased to undetectable levels (<1 ppm) and were recovered using manufacturer-recommended peroxide-quenching broth (TSB-BP16; Mesa Laboratories).

3. Results

3.1. Characteristics of HPV Smart Robot (Hydrogen Peroxide Vaporization Smart Robot System)

The HPV Smart Robot successfully completed all programmed disinfection cycles, including autonomous navigation, vapor circulation, environmental monitoring, and aeration control. Real-time monitoring confirmed attainment of the target hydrogen peroxide concentration during all robotic experiments.

3.2. Comparing the Biodecontamination Effectiveness of a Traditional HPV Disinfection System Versus HPV Disinfection with a Disinfection Robot

The conventional HPV cycle employed a 120-min injection phase and a 120-min aeration phase, whereas the robotic HPV cycles utilized shortened injection phases (90–100 min) and a fixed 96-min aeration phase under different navigation modes and fan-angle configurations (Table 1). Biodecontamination efficacy comparisons were based on the 20 environmental inoculation sites distributed throughout the enclosure. Four additional inoculation sites located within the robotic platform were evaluated separately to assess hydrogen peroxide vapor penetration into enclosed robot-internal structures.

All 20 environmental inoculation sites were successfully biodecontaminated in every experiment, including the conventional HPV cycle and all robotic HPV cycles. However, a positive biological indicator was detected at a robot-internal fan–corner junction location during the third and fourth robotic experiments (Figure 2 and Table 2), resulting in biodecontamination rates of 95–100% when robot-internal sites were included in the analysis. All environmental biological indicators demonstrated complete inactivation of *Geobacillus stearothermophilus* spores, corresponding to a ≥ 7 log₁₀ reduction from the initial inoculum (10^7 CFU). Because complete kill was observed, quantitative CFU enumeration was not possible. As expected, microbial growth was observed in the positive controls, whereas no growth was detected in the negative controls, confirming the validity of the culture conditions and microbiological recovery procedures.

In addition to microbiological performance, operational time metrics were compared between the conventional HPV system and the HPV Smart Robot (Table S1). The conventional system required approximately 15 min for setup, followed by a 120 min injection phase, a 30 min dwell phase, and a 120 min aeration phase, resulting in a total cycle time of approximately 285 min. In contrast, the robotic system required approximately 5 min for setup and employed injection phases of 90–100 min, a fixed 30 min dwell phase, and a 96 min aeration phase, resulting in total cycle times of 221–231 min depending on the operating configuration. These findings indicate that the robotic system substantially reduced setup time and overall cycle duration while maintaining high biodecontamination efficacy at all environmental sampling locations.

Table 2. Evaluation of biodecontamination effectiveness using biological indicator (BI) testing based on *Geobacillus stearothermophilus* spore growth after incubation and environmental culture sampling based on post-disinfection surface swab cultures.

No.	Specimen Types	First Experiment	Second Experiment	Third Experiment	Fourth Experiment
1	Medical cart (stainless steel)	Nil	Nil	Nil	Nil
2	Medical cart drawer (stainless steel)	Nil	Nil	Nil	Nil
3	Medical tray (stainless steel)	Nil	Nil	Nil	Nil
4	Above cabinetry (MDF)	Nil	Nil	Nil	Nil
5	In a cabinet drawer (plastic)	Nil	Nil	Nil	Nil
6	Bed sheet (cotton fabric)	Nil	Nil	Nil	Nil
7	On a medical bed mattress (leatherette)	Nil	Nil	Nil	Nil
8	Sphygmomanometer cuff (polyurethane)	Nil	Nil	Nil	Nil
9	Acrylic	Nil	Nil	Nil	Nil
10	Patient monitor handle (plastic)	Nil	Nil	Nil	Nil
11	Medical cart (plastic)	Nil	Nil	Nil	Nil
12	Chair (cotton fabric)	Nil	Nil	Nil	Nil
13	Curtain (polyester)	Nil	Nil	Nil	Nil
14	Surgical lighting supporter bottom (metal)	Nil	Nil	Nil	Nil

15	Medical cart bottom (stainless steel)	Nil	Nil	Nil	Nil
16	Operating table mattress top (arm section) (leatherette)	Nil	Nil	Nil	Nil
17	Operating table mattress top (leg section) (leatherette)	Nil	Nil	Nil	Nil
18	Medical under-bed support (metal)	Nil	Nil	Nil	Nil
19	Medical bed height adjustable footrest (rubber)	Nil	Nil	Nil	Nil
20	Chair handle (plastic)	Nil	Nil	Nil	Nil
21	HPV Smart Robot top (main body, behind FAN)	-	Nil	Nil	Nil
22	HPV Smart Robot top (main body, in front of FAN)	-	Nil	Nil	Nil
23	Inside the HPV Smart Robot (corner, FAN junction)	-	Nil	Positive	Positive
24	HPV Smart Robot circulation FAN (plastic)	-	Nil	Nil	Nil
Positive count/Total count		0/20	0/24	1/24	1/24
Biodecontamination rate (%)		100%	100%	95%	95%

Note: Nil = No bacteria detected; Positive = Bacteria detected. HPV, hydrogen peroxide vaporization; MDF, medium-density fiberboard. BI results were determined by growth/no-growth following incubation of *Geobacillus stearothermophilus* biological indicators. Environmental culture results were obtained from post-disinfection surface swab sampling and subsequent microbiological culture. Positive controls showed growth after incubation, whereas negative controls remained sterile.

3.3. Distribution and Maximum Concentration of Hydrogen Peroxide During HPV

In all robotic experiments, the target hydrogen peroxide concentration threshold (≥ 140 ppm) was achieved before completion of the programmed injection phase, supporting the feasibility of shortened robotic operating cycles. Peak hydrogen peroxide concentrations were 170 ppm in the conventional HPV cycle and 211, 204, and 224 ppm in the second, third, and fourth robotic experiments, respectively. All experimental conditions maintained concentrations above the manufacturer-recommended target threshold for effective HPV biodecontamination.

To evaluate vapor distribution, we assessed concentration variability across measurement locations. The conventional HPV system demonstrated relatively uniform distribution, with a 29-ppm difference between the highest and lowest measured concentrations. In contrast, the robotic experiments showed larger concentration gradients of 67, 78, and 101 ppm in the second, third, and fourth experiments, respectively. Consistent with these observations, the calculated coefficient of variation (CV) proxy increased from 18.6% in the conventional cycle to 37.7%, 47.3%, and 58.2% in the robotic experiments, indicating greater spatial variability in hydrogen peroxide distribution despite maintenance of concentrations above the effective threshold.

Chemical indicator results demonstrated a different distribution pattern. In the conventional HPV cycle, incomplete chemical indicator color change was observed at the fumigator location (site 7) and the stainless-steel drawer (site 21), both representing relatively sheltered locations within the enclosure. In contrast, all chemical indicators changed appropriately in the second and third robotic experiments. During the fourth experiment, incomplete indicator responses were observed at the inner corners (sites 3–4), the fumigator location (site 7), and locations between the door and air conditioner (sites 8–12) (Figure 3).

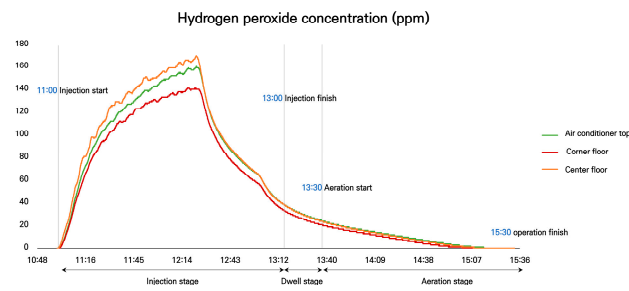
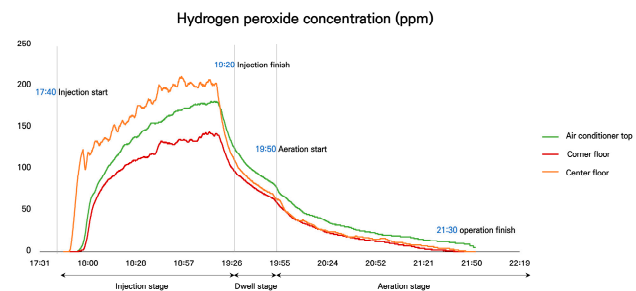
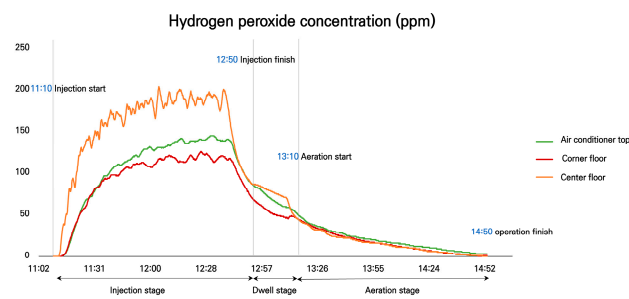
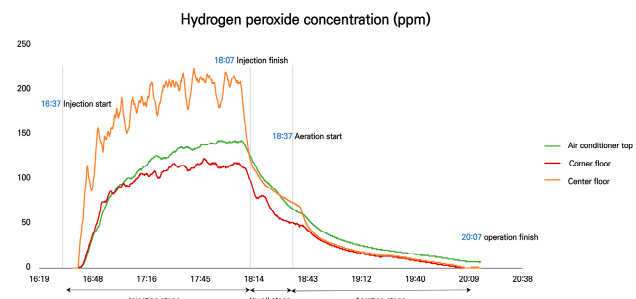
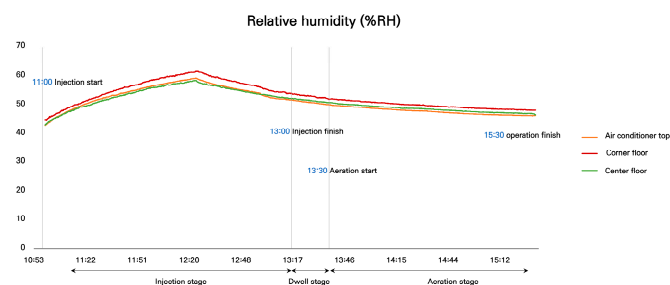
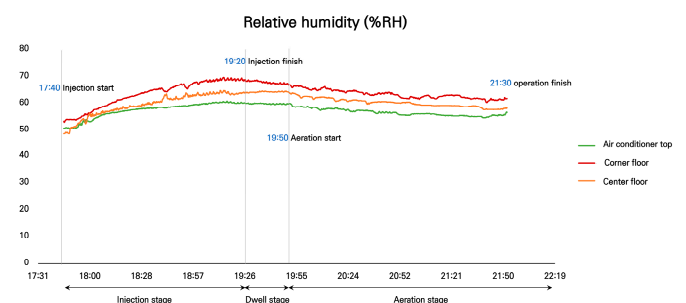
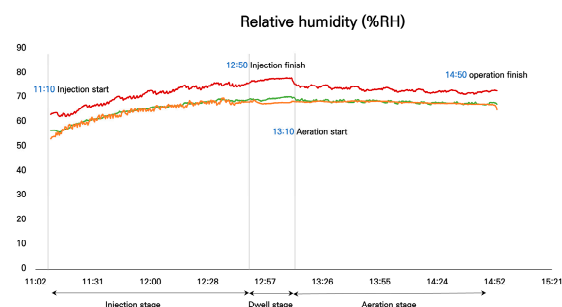
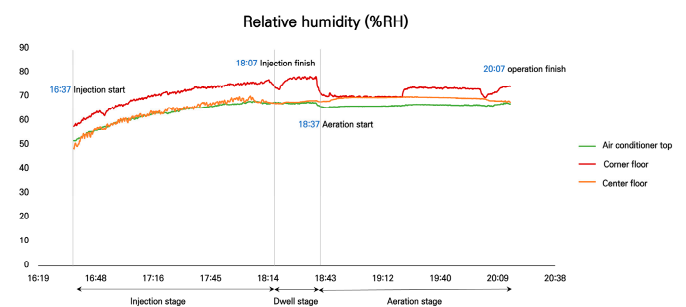
a**1st experiment (Traditional methods using 'Huen IVH')****2nd experiment (mobile sterilizer + robotic application)****3rd experiment (mobile sterilizer + robotic application)****4th experiment (mobile sterilizer + robotic application)****b****1st experiment (Traditional methods using 'Huen IVH')****2nd experiment (mobile sterilizer + robotic application)****3rd experiment (mobile sterilizer + robotic application)****4th experiment (mobile sterilizer + robotic application)**

Figure 3. Hydrogen peroxide distribution concentration change and humidity change. (a) Change in hydrogen peroxide distribution concentration; (b) humidity changes in the clinical trial enclosure.

To further characterize concentration stability, mean hydrogen peroxide concentrations, standard deviations (SD), and 95% confidence intervals were calculated from plateau-phase measurements after attainment of the target concentration (Table S2). Mean

concentrations ranged from 155.5 to 177.5 ppm across all experimental conditions, exceeding the target threshold in every cycle. Variability was lowest in the conventional HPV cycle (SD 7.64 ppm) and higher in the robotic experiments (SD 10.05–24.64 ppm). Because these measurements represented repeated spatial observations within individual experimental cycles rather than independent samples, the results are presented descriptively rather than as formal statistical comparisons. Although spatial variability was greater in the robotic experiments, all systems achieved high biodecontamination efficacy (95–100%), with hydrogen peroxide concentrations remaining within ranges considered effective for microbial inactivation.

3.4. Changes in Hydrogen Peroxide Concentration and Humidity in the Enclosure

During the aeration phase following the HPV process, a transient increase in hydrogen peroxide concentration was observed due to the difference in vapour pressures between water and hydrogen peroxide. The residual vapour was subsequently decomposed and removed through the scrubber system. The aeration efficiency of hydrogen peroxide was similar across the four experiments, and at the end of all experiments, hydrogen peroxide was degraded to undetectable levels. However, there was a slight difference in aeration time: the standard HPV method required 120 min for complete scrubbing, whereas the vaporization biodecontamination robot achieved it within an average of 96 min.

After aeration ended, the highest humidity in the enclosure was 61.6% vs. an average of 75.3%. However, there may be some bias because the base humidity was 42.9% at the beginning of the experiment (the first experiment). In contrast, as the experiment progressed, the base humidity increased to 48.6%, 53.2%, and 48.5%, respectively (Figure 3).

4. Discussion

Biodecontamination is a critical component of infection prevention strategies aimed at reducing healthcare-associated infections (HAIs). Although hydrogen peroxide vapor (HPV) is an established high-level disinfection modality, conventional workflows remain labor-intensive and time-consuming, underscoring the need for more efficient and standardized approaches [29]. In recent years, robotic disinfection systems have gained increasing attention as adjuncts to manual cleaning, particularly UV-based platforms that have demonstrated significant reductions in environmental microbial contamination [19,30,31]. However, real-world evaluations have revealed persistent limitations, including shadowing effects and reduced efficacy in complex hospital layouts [32]. Other robotic disinfection approaches have also incorporated chemical spray systems, such as chlorine dioxide, and cost-effective UVC-based platforms [33,34].

Against this background, the present study was designed to conduct a preliminary comparative performance evaluation of the HPV Smart Robot system relative to a conventional HPV disinfection system under controlled environmental conditions. The robotic HPV system achieved complete biodecontamination at all 20 environmental sampling locations and demonstrated overall biodecontamination rates of 95–100% when robot-internal sites were included in the analysis. Compared with UV-based robotic systems, which are constrained by line-of-sight dependence and lower sporicidal activity, hydrogen peroxide-based technologies provide superior penetration into shadowed areas and greater activity against resistant spores, supporting HPV as a robust modality for robotic automation.

Beyond microbiological performance, the HPV Smart Robot provided important operational advantages. The robotic platform integrates autonomous navigation, adjustable fan-angle configurations, and real-time environmental monitoring, reducing the need for manual equipment placement and room preparation. Setup time was reduced from approximately 15 min to 5 min, and total cycle duration was shortened by 54–64 min com-

pared with the conventional workflow. These findings suggest the potential for improved staff efficiency and room turnover while reducing occupational exposure risks associated with environmental disinfection procedures. However, formal cost-benefit and quantitative labor-saving analyses were not performed and warrant future investigation.

A positive biological indicator was detected at a recessed fan-corner junction within the robotic platform, likely reflecting localized airflow limitation and reduced vapor penetration into an enclosed internal structure. Although complete biodecontamination was achieved at all environmental sampling locations, residual contamination within a robot-internal component could theoretically serve as a contamination-retention site if similar conditions occur during repeated clinical use. These findings highlight the importance of airflow optimization, fan-angle configuration, and navigation-path refinement when developing robotic HPV systems. Future robotic platforms should incorporate design features that facilitate vapor penetration into recessed internal structures and allow routine inspection, cleaning, and maintenance of enclosed components.

The apparent discrepancy between biological indicators and environmental culture results warrants consideration. Biological indicators containing highly resistant *Geobacillus stearothermophilus* spores are widely regarded as the reference standard for validating sporicidal efficacy and demonstrated complete inactivation under nearly all tested conditions. In contrast, environmental culture sampling reflects microbial recovery from specific locations and may be influenced by local airflow patterns, surface geometry, and vapor penetration characteristics. Therefore, the isolated positive biological indicator observed at the fan-corner junction most likely represents localized residual contamination within a difficult-to-reach microenvironment rather than failure of the overall HPV biodecontamination process. Together, these findings highlight the complementary roles of biological indicators and environmental culture methods in assessing both global sporicidal performance and site-specific decontamination effectiveness.

Hydrogen peroxide concentration measurements demonstrated greater spatial variability during robotic operation than during conventional HPV disinfection. This concentration gradient is likely attributable to the strong airflow generated by the robotic circulation fan, as the system was designed to prioritize broad environmental coverage rather than strict concentration uniformity. Despite this variability, all measured concentrations remained above the manufacturer-recommended target threshold (≥ 140 ppm), and high biodecontamination efficacy was maintained across environmental sampling locations. Additional optimization of airflow strength, fan-angle configuration, and navigation pathways may further improve concentration uniformity while preserving effective microbial inactivation.

From an infection prevention and control (IPC) perspective, safety remains a critical consideration for HPV deployment. Residual hydrogen peroxide concentrations were continuously monitored during aeration, and all experimental cycles achieved safe re-entry levels (<1 ppm) before room access, consistent with international occupational safety recommendations. Furthermore, all experiments were performed in unoccupied environments, and no visible surface damage or equipment impairment was observed following repeated HPV exposure.

Limitations

This study has several limitations. First, all experiments were conducted in a controlled simulation enclosure rather than in active hospital wards, which may not fully reflect real-world clinical environments, including complex room geometries, ventilation patterns, workflow integration, and cluttered room layouts commonly encountered in healthcare settings.

Second, only one conventional HPV cycle and three robotic HPV cycles were evaluated, with differences in navigation mode, fan-angle configuration, and baseline environmental conditions, including relative humidity. Because relative humidity can influence hydrogen peroxide vapor distribution, condensation behavior, microbial inactivation efficiency, and aeration dynamics, the observed differences in vapor concentration profiles and operational times may have been affected by both system characteristics and environmental variability. Therefore, the present findings should be interpreted as exploratory comparative performance data rather than results from a fully replicated comparative trial, and direct attribution of observed differences solely to system type should be made with caution.

Third, standardized *Geobacillus stearothermophilus* biological indicators were used as the primary microbiological challenge. Although these indicators represent a conservative and widely accepted method for validating sporicidal efficacy, they do not permit quantitative assessment of log₁₀ reductions or direct extrapolation to clinical surface contamination scenarios. Furthermore, biological indicator outcomes were assessed qualitatively as growth or no growth after incubation, and quantitative CFU enumeration was not performed. Therefore, the residual microbial burden at the positive robot-internal location could not be quantified.

Fourth, limitations attributed to conventional HPV systems are specific to the tested Huons platform and may not be generalizable to other HPV or VHP systems employing different vaporization, distribution, or aeration technologies.

Finally, formal cost–benefit analyses and quantitative assessments of labor savings were not performed. In addition, because this study was not conducted as a formal conformity assessment according to EN 17272 [27], the findings should be interpreted as comparative performance data rather than certification-level validation.

5. Conclusions

In conclusion, the HPV Smart Robot demonstrated high microbiological efficacy and operational feasibility under the experimental conditions evaluated in this study. Future multi-center longitudinal studies incorporating economic modeling, time–motion analyses, and long-term clinical outcome assessments are warranted, along with investigations into hybrid technologies combining hydrogen peroxide vapor and UV light and evaluations of user acceptance and training needs to facilitate safe, cost-effective, and sustainable integration into routine clinical workflows.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/app16147035/s1>, Table S1: Operational time metrics for conventional and robotic HPV methods, Table S2: Hydrogen peroxide concentration variability metrics.

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Conflicts of Interest: The HPV Smart Robot was developed for research purposes only, without any commercial or financial interests. The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

BI	Biological Indicator
CFU	Colony-forming unit
HAIs	Healthcare-associated Infections
HPV	Hydrogen Peroxide Vaporization
IPC	Infection Prevention and Control
TSB	Tryptic Soy Broth
UV	Ultraviolet

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