








Public Buses Decontamination by Automated Hydrogen Peroxide Aerosolization System

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Abstract

BACKGROUND: Public transportation has been linked to an increase in the risk of coronavirus disease 2019 transmission. The effective decontamination system using aerosolized hydrogen peroxide can mitigate the transmission risk from using public transportation.

AIM: The aim of this study was to develop and validate an effective decontamination system for public transport.

METHODS: The experimental research was performed in 13 inter-city public buses. The aerosol generator with ultrasonic atomizer was used in the experiment. The validation process for disinfection was conducted using both a chemical indicator (CI) and spore discs biological indicator (inoculated with 10^6 *Geobacillus stearothermophilus* enclosed in glassine envelopes). The CIs and biological indicators were marked by number and placed in nine locations on each bus. The decontamination cycle was developed by analyzed of various aerosolized and decomposition period. Both concentrations of hydrogen peroxide, 5% and 7%, were used for comparison.

RESULTS: In an aerosolized period, both concentrations of hydrogen peroxide at 30 min were effective for sporicidal 6-log reductions. The decontamination cycle totaled 100 min, based on a 70 min average decomposition time.

CONCLUSIONS: The automated hydrogen peroxide aerosolized system is a highly effective and safe method of decontaminating public buses.

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Introduction

The global coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), originated in Wuhan (China) in December 2019 and has spread to over 200 countries. Most countries use preventive measures to control infection transmission, including physical distancing, wearing a face mask, prohibit crowd gathering, frequently cleaning hands with soap or alcohol-based hand sanitizers, social distancing, travel restriction, and lockdown. These counter measures have affected various industries and sectors in several ways. Public transport was also heavily affected by the outbreak. Several measures for public transportation, including advice to avoid public transport, and reduce the number of passengers to allow

physical distancing, have led to an expected dramatic decrease in ridership (40–90%) during the early period of the outbreak [1], [2], [3], [4]. The fear of being infected has resulted in reduced mobility and avoidance of public transport [5]. Zhang *et al.* [6] found that the largest share of public transport modals shifted to private cars during the pandemic. This decline in ridership has caused the financial instability of transit operators [7], [8], [9]. These effects not only affect behavioral change but also attitudes toward public transport. Some studies have expressed the concern of public returning to public transportation in post-COVID-19 [10], [11].

Public transport has been debated as an increased risk of COVID-19 transmission due to its confined spaces, limited ventilation, and prolongs duration time. The case report of the infection clusters on

a tour bus in China with the index case that transmitted the virus to 23 of a total of 68 passengers [12]. Similarly, observations have been reported in another public bus with an index case transmitted to other ten people [13]. In addition, in-flight transmission has been reported; for example, 12 people were infected on a 325-passenger flight from Singapore to Zhenjiang, China [14], and 16 out of 217 passengers were infected on a flight from London to Hanoi, Vietnam [15].

The route of COVID-19 transmission is through droplet, fomite contact, and airborne transmission under special circumstances [16]. Fomite transmission is an indirect contact that occurs when non-infected people touch contaminated surfaces with their hands and subsequently touch their mouth or nose. Under certain conditions, fomite transmission can spread widely and rapidly. The transmission of pathogens by contaminated surfaces to the hands can be spread up to 14 individuals and by hand-to-hand sequentially up to six individuals [17]. A modeling study of the spread ability by fomite transmission in aircraft showed that most of the high-touch surfaces in the cabin were contaminated within 2 or 3 h [18]. Many studies have found environments contaminated with SARS-CoV-2 in both health-care settings [19], [20], [21], [22], [23] and non-health-care settings [24], [25], [26], [27], [28], [29]. The evidence showed that SARS-CoV-2 could persist on surfaces for several hours or days [30]. There have been reports of COVID-19 infection linked to fomite transmission [31], [32]. However, some researchers suggest that fomite transmission is a low risk, as most studies of environmental contamination do not present the real-life situation [33], [34]. Only SARS-CoV-2 RNA has been detected, with only few studies successful in culturing viable viruses from surfaces [35]. At present, there is limited evidence on the proportion of fomite transmission in SARS-CoV-2 infection. However, the new strains of SARS-CoV-2 have been shown to possess better transmissibility and may behave differently in fomite transmission [36]. Moreover, fomites play an important role in the transmission of certain bacteria and viruses (e.g., Methicillin-resistant *Staphylococcus aureus*, SARS-CoV, and Norovirus) [37], [38], [39], [40].

A safe strategy for mitigating the risk of fomite transmission is implementing surface cleaning and disinfection. The researchers found that city disinfection was an important strategic policy to help prevent the spread of COVID-19 [41]. Surface cleaning and disinfection can be performed either by traditional manual methods or modern automated methods. There is strong evidence indicating that traditional cleaning and disinfection methods are not adequate for infection prevention and control. According to the previous studies, traditional methods only cover 40–60% of the surfaces that should be cleaned [42], [43]. To improve cleaning and disinfection, particularly in health-care settings, automated technologies are recommended to supplement traditional methods [44]. These modern

technologies cannot be used in place of traditional methods because a traditional method is necessary to eliminate the visible dirt. Numerous technologies have been developed in an attempt to increase the cleaning and disinfection coverage area, such as spraying or fumigating disinfectants. Spraying of commonly used disinfectants such as hypochlorite-based products and quaternary ammonium compounds (Quats) is considered an effective method of microbial decontamination. However, the study discovered that trigger spray and electrostatic spray still had coverage area limitations [45]. Spraying disinfectant is ineffective at removing contaminants outside of direct spray zone [46]. In addition, spraying disinfection systems require human control, which could increase the risk of developing asthma and respiratory tract disease to users. Benzalkonium chloride (BAC) is one of the Quats common used for surface disinfection. The study found that BAC is not degraded rapidly and its antimicrobial effect last on the surface for days [47]. The residual of BAC even low concentration could cause skin irritation [48]. Gas fumigation such as formaldehyde and chlorine oxide is highly effective antimicrobial agents. However, these disinfectants pose health risks of cancers and respiratory diseases [49]. In a cost comparison of disinfectants with the same high-disinfection level efficiency, 7.5% hydrogen peroxide is found to be less expensive than 0.2% peracetic acid, 2% glutaraldehyde, and 0.55% ortho-phthalaldehyde when using with manual or automated methods [50].

Two of the most frequently used automated technologies are the ultraviolet germicidal irradiation (UVGI) system and the hydrogen peroxide (H_2O_2) system. The UVGI system is well studied and used for air and surface disinfection in hospitals. The advantage of UVGI is that it is simple to use and leaves no residue, but the main limitation is that shadowing can result in a decrease in disinfection efficacy. Hence, in the complex room with potential shadow, H_2O_2 is preferred to the UVGI system [51]. Public buses contain areas that are out of sight, such as the area beneath the seat. The H_2O_2 system appears to be suitable for decontaminating public buses. In addition, H_2O_2 decomposes spontaneously into oxygen and water, which are non-toxic byproducts. New research has focused on decontamination methods that are both effective and environmental friendliness [52], [53].

There are two types of H_2O_2 systems: A hydrogen peroxide vapor (HPV) system and an aerosolized hydrogen peroxide (aHP) system. The aHP system is also known by the alternative name “dry mist hydrogen peroxide,” which is misleading in terms of its properties [54]. Two typical HPV technology systems are the dry process (Steris Corporation, Ohio, and USA) and the wet process (Bioquell, Hampshire, and UK). The dry process operates without condensation and requires humidity control before operation. In general, the decontamination cycle for a dry process consists of four phases by (1) dehumidification, where

relative humidity is reduced; (2) conditioning, where the vapor is released until reached desire concentration; (3) decontamination, where the vapor is steady released to keep the constant concentration, and (4) aeration, where the residual H_2O_2 is removed by a catalytic process. By contrast, the wet process does not require humidity control and therefore result in micro-condensation. However, the decontamination cycle is similar to dry system which consists of four phases: Conditioning, gassing, dwell, and aeration. The difference from the dry process is that no vapor is emitted during dwell phase, this permitting the peroxide to dwell on any surface exposed. Both HPV systems are produced by heating 30–35% liquid H_2O_2 to a vapor, while aHP uses a low concentration of H_2O_2 (5–7%) by pressure or ultrasonic injection. Another distinction is the process of H_2O_2 decomposition: HPV is the active aeration phase, while aHP is the passive phase in which the left aerosol decomposes naturally. According to their disinfection efficacy, the HPV system has been supported by many studies [55], [56], [57], while the use of the aHP system has been limited. Several studies have compared the effectiveness of HPV disinfection systems to aHP systems, indicating that HPV systems are more effective at disinfecting than aHP systems, despite concerns about the reliability of aHP systems [58], [59], [60]. On the contrary, Ali *et al.* [61] demonstrated that aHP decontamination was successful at inactivating pathogens and was comparable to HPV systems. HPV disinfection systems are widely used for disinfecting isolators, medical equipment, and room disinfection. By comparison, aHP disinfection system provides greater containment of H_2O_2 within an area of application [62], potentially eliminating the need to seal the door and window edges of the room. As a result, rooms that were not originally designed for vapor systems could be used with aHP [63]. Further, aHP has lower machine and maintenance costs and can be easily scaled.

H_2O_2 has long been used as a disinfectant and antiseptic. There have been few studies that using low H_2O_2 concentration for ingestion or breathing to treat diseases including such COVID-19 [64]. However,

high levels of exposure, ingestion, or breathing can be extremely dangerous. Drinking high concentrations of H_2O_2 can be fatal or cause serious harm. Five people reported chest pain, stomach pain, difficulty breathing, and loss of consciousness after drinking 50 mL of 33% H_2O_2 [65]. The US FDA has warned that drinking 35% H_2O_2 can cause gastrointestinal irritation or ulceration which may lead to death [66]. For inhalation, H_2O_2 concentration level <1 ppm is considered safe. Longitudinal studies of a worker exposed to H_2O_2 at concentrations <1 ppm revealed no effect on lung function [67]. However, inhalation of H_2O_2 at a concentration of 2.2 ppm for 2 h was found to cause mild irritation, including nasal airway resistance.

The purpose of this study was to validate the disinfectant efficacy of aHP and to develop a decontamination cycle for public buses. Throughout the decontamination process, the concentration of residual H_2O_2 was monitored to ensure that it was <1 ppm at the end of the cycle. The permissible exposure limit of H_2O_2 , according to the Occupational Safety and Health Administration, is 1 ppm for an average of 8-h time-weighted averages [68]. H_2O_2 concentrations of <1 ppm would allow people to reenter the area in which it is being used.

Materials and Methods

Aerosolized generator and disinfectants

The ultrasonic aerosol generator with an injection rate of 20 mL/min was used in the experiment. The generator features pre-set timing for the aerosolized period and delayed time prior release aerosol for safety to operators. The generator was placed in the center of the bus and was pre-set with different aerosolized period 16, 20, 25, and 30 min. The windows and doors remain closed during the decontamination cycle. The two different concentrations of H_2O_2 were used to

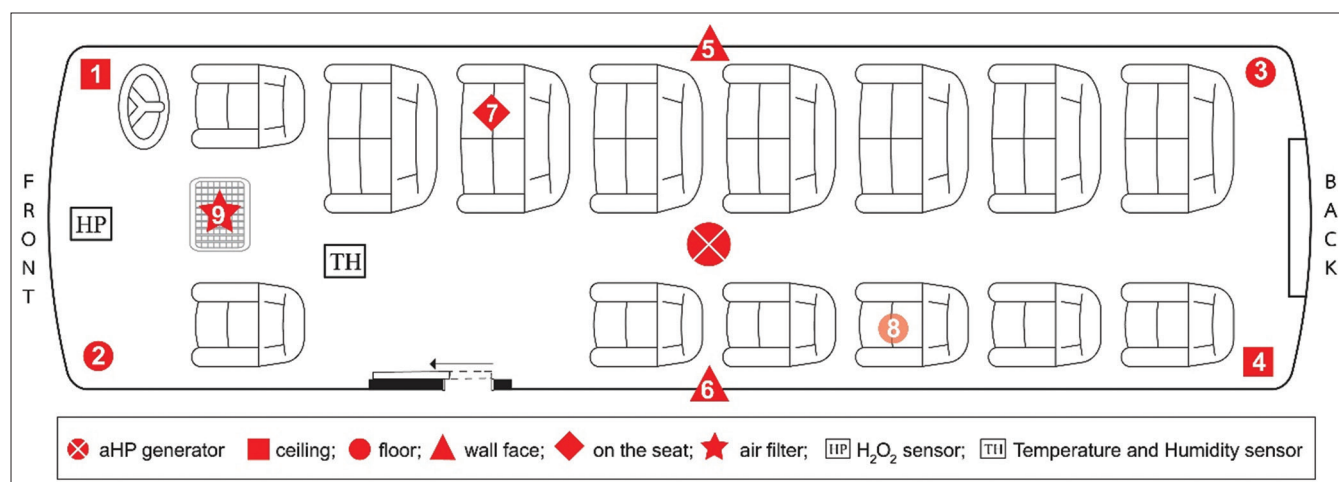


Figure 1: The location of chemical indicators and biological indicators placement inside the public buses

compare between 5% H₂O₂ and 0.005% silver ions (Sanosil S010 and Sanosil AG) and solutions prepared by diluting 50% H₂O₂ food grade (Interox FG50 and Solvay Thailand) with deionized water to a final concentration of 7% H₂O₂ solution.

Decontamination process and validation

The temperature and humidity were measured by a handheld meter placed inside the bus during the decontamination process (GSP-6, Elitech Technology Inc, USA, accuracy $\pm 0.5^{\circ}\text{C}$, and $\pm 3\%$ RH). The H₂O₂ sensor meter (CB-100, Membrapor AG, Switzerland, and accuracy $\pm 3\%$), with a range of 0–20 ppm, was placed inside the bus by the window to measure the residual H₂O₂. The duration after aerosolized period until H₂O₂ concentration is below 1 ppm is considered to be the decomposition time which indicates that the decontamination process had ended. To validate the disinfection efficacy, chemical indicator (CI) strips (Comply™ Hydrogen Peroxide CI 1248, 3M, USA) and biological indicator (BI) envelopes containing 2.30×10^6 *Geobacillus stearothermophilus* ATCC 7953 dried on stainless-steel metal discs sealed in glassine paper (Sterind Bio-indicator *G. stearothermophilus*, Micro

Biotech Inc., India) were marked by number and placed in hard-to-reach locations inside the buses on the floor, ceiling, wall faces, on the seat, and beneath the seat (Figure 1). At the end of the process, CIs and BIs were evaluated. A CI that changed its color from blue to pink was considered exposed to H₂O₂ and reported as a positive test. The BI was retrieved, and the envelope was opened. A disc was transferred to culture in Tryptic soy broth at 56°C for 7 days. Turbidity was observed for re-growth of the spores which indicating failure or positive test.

Public buses

This study was performed in 13 buses at the public bus station of Trat City (the eastern province of Thailand). The 20 seater air-conditioned bus with dimension 2 m×6.7 m× 1.9 m (W×D×H), an interior volume of 25.46 m³ was employed. The buses operated between Trat and Bangkok, with a distance 320 km. Bangkok is the capital of Thailand and Trat is the major attraction tourist city. The total transit duration was approximately 6 h, including stops at six locations along the route (Figure 2).

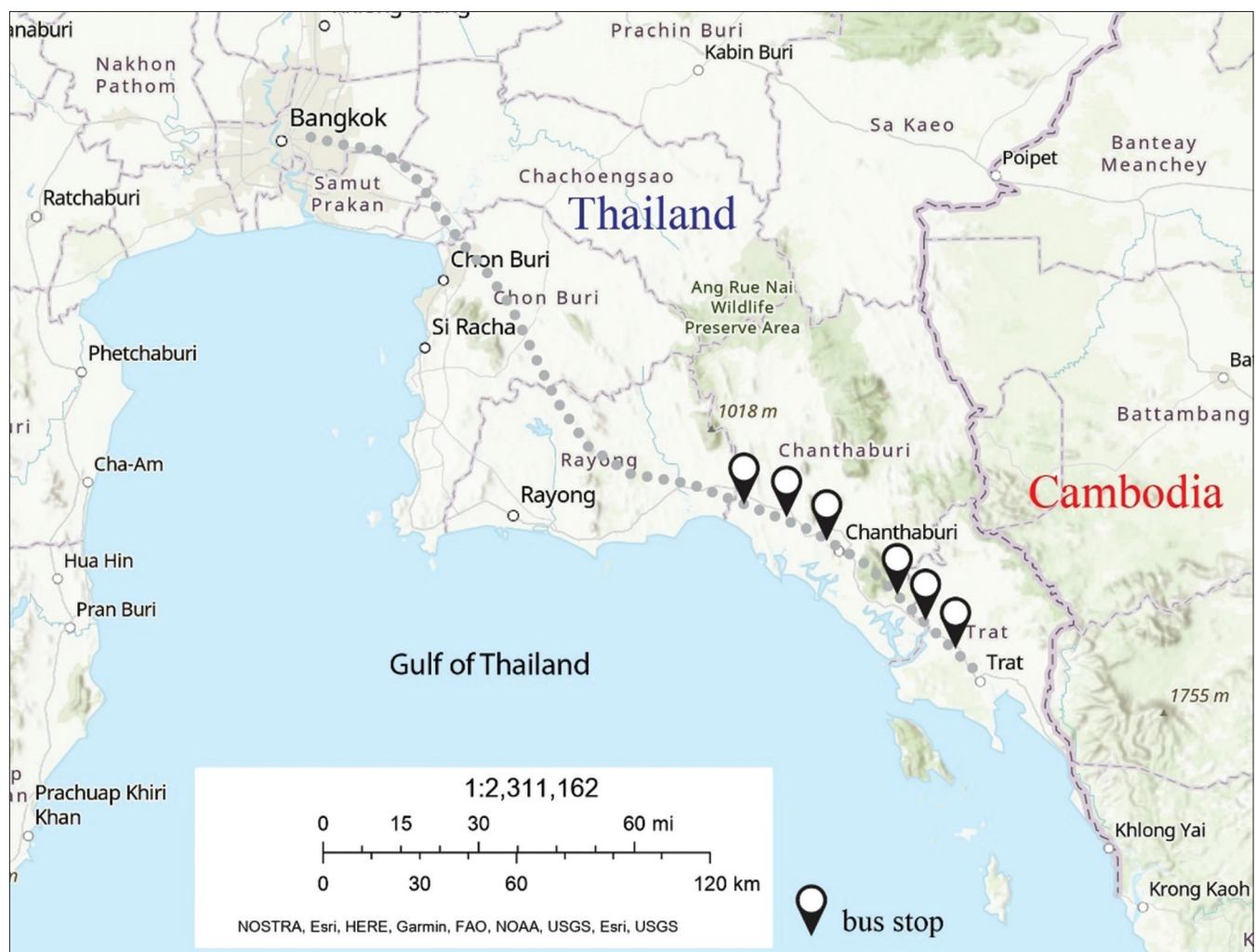


Figure 2: Inter-city bus route between Trat and Bangkok

Results

During aerosol injection, the relative humidity increased, and the temperature decreased until the aerosolized period was complete, at which point the relative humidity decreased (Figure 3). H₂O₂ concentrations increased during the decontamination process, and condensation was observed near the location of the generator. Each bus had nine CIs and BIs, for a total of 117 CIs and 117 BIs.

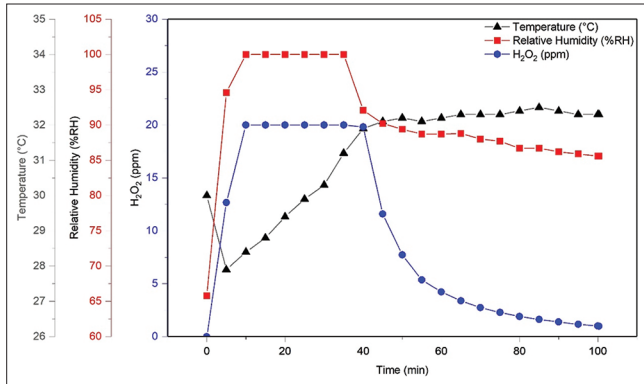


Figure 3: Temperature, relative humidity, and hydrogen peroxide concentration during decontamination cycle with 30 min aerosolized period

All CIs (100%) were fully exposed to H₂O₂, while 89 (76.06%) of the 117 BIs demonstrated effective decontamination. At 16 min, the aerosolized period was insufficient to disinfect all BIs. Exposure times of 20 and 25 min were insufficient to inactivate some BI locations (Table 1).

The exposure time of 30 min revealed effective inactive BIs at all locations, with either 5% or 7% H₂O₂ concentrations. Decomposition time of 70 min (95% CI; 47–86 min) combined with an exposure time of 30 min yielded a total of nearly 2 h as an effective decontamination cycle for the 20-seater bus.

Discussion

H₂O₂ is a potent oxidizing agent. It is broad-spectrum microbial activity against bacteria, viruses,

molds, and spores by generating free hydroxyl radicals, leading to the oxidation of the lipid membranes, protein, and deoxyribonucleic acid of microorganisms. H₂O₂ in the vapor phase is a more potent protein oxidizer than H₂O₂ in the liquid phases [69].

In this study, we discovered that when the aerosol was injected, the relative humidity increased and remained plateaued until the injection was complete, at which point it decreased. Both the aHP and HPV systems can raise relative humidity during the aerosolized period. There have been two disagreements about the effect of humidity on the decontamination efficiency of both H₂O₂ systems [70]. High relative humidity has a negative impact on the decontamination efficiency of the HPV system, as areas with high humidity cannot contain high concentrations of H₂O₂. Condensation occurs when the vapor concentration of H₂O₂ is higher than its saturation level, which inhibits the homogenous distribution of the vapor. Thus, in a dry process system, the relative humidity has to be reduced prior to decontamination with H₂O₂. Dehumidification before using an aHP system has been shown to improve decontamination performance. In another point of contention, the efficiency of surface disinfection is dependent on condensation on the surface being decontaminated. High humidity has a greater impact on disinfection efficacy than low humidity. Therefore, in the wet process, humidity control before decontamination is not required. However, some studies have indicated that the humidity factor is a less important factor and that it may not be necessary to pre-condition by reducing humidity prior to decontamination [71]. In this study, no humidity control was performed before decontamination. The experiment was conducted in a real-life situation. The H₂O₂ sensor had a maximum reading of 20 ppm, but our objective was not to determine the relationship between the concentration and decontamination efficacy. The sporicidal efficacy resulted in a 6-log reduction. This study demonstrated the effectiveness of aHP in real-world scenarios. The lack of humidity control emphasizes the advantages of aHP over other systems in terms of ease of use.

According to the International Organization for Standardization (ISO) 11139:2018, a CI is a “test system that reveals change in one or more pre-specified process variables based on a chemical or physical

Table 1: Results of aHP decontamination by chemical indicators and biological indicators at various aerosolized periods

Bus number	Source of H ₂ O ₂	Aerosolized period (min)	CI locations									BI locations								
			1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
#1	5% H ₂ O ₂ +0.005%Ag	30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
#2	7% H ₂ O ₂	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
#3	7% H ₂ O ₂	16	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
#4	5% H ₂ O ₂ +0.005%Ag	30	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
#5	5% H ₂ O ₂ +0.005%Ag	30	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
#6	7% H ₂ O ₂	30	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
#7	7% H ₂ O ₂	30	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
#8	7% H ₂ O ₂	20	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	+	+	
#9	7% H ₂ O ₂	25	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	
#10	7% H ₂ O ₂	20	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	
#11	7% H ₂ O ₂	25	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	+	
#12	7% H ₂ O ₂	30	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	
#13	7% H ₂ O ₂	30	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	

change resulting from exposure to a process,” while the BI is a “test system containing viable microorganisms providing a specified resistance to a specified sterilization process” [72]. ISO 11138-1:2017 guides the selection and use of BIs for ethylene oxide, moist heat, dry heat, and low-temperature steam formaldehyde but not for HPV and aHP. For the US FDA 510k, a BI is needed to validate the terminal sterilization process of medical devices by HPV with the recommended strain of *G. stearothermophilus* [73]. The finding that *G. stearothermophilus* is the most resistant microorganism to H₂O₂ is supported by research [74]. Several studies have suggested using *G. stearothermophilus* spores to validate the aHP process [75], [76]. We used 2.30 × 10⁶ *G. stearothermophilus* ATCC 7953 to represent the most resistant strain to the aHP decontamination process and placed BIs in nine locations for each bus in accordance with the room sterilization protocol by the US Environmental Protection Agency (US EPA) that recommends for testing BI must contain ≥10⁶ *G. stearothermophilus* (ATCC 7953) spores, and test location of BIs must include all corners of the rooms, wall faces, center location, and underneath horizontal surfaces [77]. In our study, all CIs were exposed to H₂O₂ at all locations, but this did not guarantee sporicidal efficacy, as determined by BIs. However, CIs will continue to be required to validate the decontamination efficacy since they can be analyzed immediately following the completion of the decontamination process, while BIs require 5–7 days to be interpreted.

In our study, we achieved a 6-log reduction in sporicidal efficiency, which is equivalent to sterilization. Roberts [82] show that 6-log sporicidal sterilization is more suitable for the terminal sterilization of medical devices compared to room decontamination because ambient surface contamination with microorganisms rarely exceeds a 2-log concentration. To challenge a lower log reduction, the amount of H₂O₂ used, the time required for decontamination, and the turnaround time can be decreased. For public bus services, turnaround time is critical. Due to the need for a total of 2 h of decontamination, it may be reasonable to perform the decontamination at the end of the day. However, shortened decontamination cycle can be operated in daytime during transfer. The aim of this study was to determine the effectiveness of decontamination using a worst-case scenario that could be beneficial during an outbreak. The outcome, which demonstrated high effectiveness, was also beneficial in re-establishing confidence in public transportation.

For the validation and cycle development of the aHP process, we conducted three repetitive tests to demonstrate the reliability of the process. We used only one cycle in contrast to other studies that have found that three cycles of aHP are needed effectiveness (Table 2). In our study, we used both commercial 5% H₂O₂ + 0.005% Ag and 7% H₂O₂ concentrations. They were both found to be successful but with different

costs, as the cost of the prepared 7% solution was 10-fold lower than that of the commercial solution. Given that during the pandemic, public bus operators struggled with a lack of revenue and higher disinfectant costs due to the increased frequency of cleaning and disinfection, a low-cost decontamination process with aHP may be preferable.

Table 2: The studies of room and vehicle decontamination

Study	Decontamination systems	Setting	Validations test	Results
Andersen <i>et al.</i> 2006 [78]	aHP	ambulance	2.5×10 ⁶ <i>Bacillus atrophaeus</i> biological indicator	three cycles of aHP achieved 6-log spores reduction. Total decontamination time is 4–6 h
Havill <i>et al.</i> 2012 [51]	Hydrogen peroxide vapor and UVC	patient room	1×10 ⁶ <i>Geobacillus stearothermophilus</i> biological indicator	Hydrogen peroxide vapor was more effective than UVC, with a 6-log spores reduction versus a 2-log spores reduction
Kostyuchenko <i>et al.</i> 2009 [79]	UVC	metro public transport system	Suspension of <i>Staphylococcus aureus</i>	A high dose of UV was required (4500 J/m ²) for 99% disinfection
Klaus <i>et al.</i> 2016 [80]	Formaldehyde, hydrogen peroxide, and alcohol	Aircraft	Not performed	Laboratory tests have revealed that these disinfectants were effective and compatible with aircraft components
Lindsley <i>et al.</i> 2018 [81]	UVC	ambulance	3–4×10 ⁷ <i>Bacillus subtilis</i> as a surrogate for pathogens	The disinfection efficacy was varying on ultraviolet germicidal irradiation fixture position. Based on these results, a UVC dose of 52.6 mJ/cm ² was required to inactivate 99.9% of the spores on a coupon

The limitation of this study is that it was conducted on a single-size vehicle. A bigger vehicle may require other parameters such as a longer decontamination cycle or the usage of more than one aHP generator, or additional factors as installing fans for assisted distribution of aerosol. During the study, we observed the compatibility of materials, such as fabric seats or interior paint, and we did not find significant issues. Since we conducted this study over a short period, further long-term research is necessary to investigate the material compatibility of the public buses.

Conclusion

The highly effective disinfection of public transportation with aHP that achieves a sporicidal 6-log reduction can help reduce the risk of infectious disease transmission. The 100 min decontamination cycle creates the potential for widespread use in public transportation that demands quick turnaround.

Automated H₂O₂ aerosolization system has many advantages, including ease of use, environmental friendliness, versatility, reliability, low cost of machine, and operations. The important point is that validations are required prior to actual use in any area or room. Validation is based on the effectiveness of decontamination and turnaround time. The automated system is safe for the workers because it does not require human beings to operate and contributes to the reduction of the effects of human error.

This decontamination procedure is applicable to public buses, but it must be emphasized that it is a supplement and cannot be used in place of cleaning. Enhancing the disinfection of public buses can help mitigate the risk of infectious transmission and regain trust in public transportation during and after a pandemic. This is the “next normal” in which public transport operators compete for sanitation to gain and retain regular passengers.

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