Efficacy, efficiency and safety aspects of hydrogen peroxide vapour and aerosolized hydrogen peroxide room disinfection systems

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SUMMARY

Background: This was a head-to-head comparison of two hydrogen-peroxide-based room decontamination systems.

Aim: To compare the efficacy, efficiency and safety of hydrogen peroxide vapour (HPV; Clarus R, Bioquell, Andover, UK) and aerosolized hydrogen peroxide (aHP; SR2, Sterinis, now supplied as Glosair, Advanced Sterilization Products (ASP), Johnson & Johnson Medical Ltd, Wokingham, UK) room disinfection systems.

Method: Efficacy was tested using 4- and 6-log *Geobacillus stearothermophilus* biological indicators (BIs) and in-house prepared test discs containing approximately 10^6 meticillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* and *Acinetobacter baumannii*. Safety was assessed by detecting leakage of hydrogen peroxide using a hand-held detector. Efficiency was assessed by measuring the level of hydrogen peroxide using a hand-held sensor at three locations inside the room, 2 h after the start of the cycles.

Findings: HPV generally achieved a 6-log reduction, whereas aHP generally achieved less than a 4-log reduction on the BIs and in-house prepared test discs. Uneven distribution was evident for the aHP system but not the HPV system. Hydrogen peroxide leakage during aHP cycles with the door unsealed, as per the manufacturer’s operating manual, exceeded the short-term exposure limit (2 ppm) for more than 2 h. When the door was sealed with tape, as per the HPV system, hydrogen peroxide leakage was <1 ppm for both systems.

The mean concentration of hydrogen peroxide in the room 2 h after the cycle started was 1.3 (standard deviation (SD) 0.4) ppm and 2.8 (SD 0.8) ppm for the four HPV and aHP cycles, respectively. None of the readings were <2 ppm for the aHP cycles.

Conclusion: The HPV system was safer, faster and more effective for biological inactivation.

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Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii* and *Clostridium difficile* are important causes of healthcare-associated infection. These three pathogens are shed by infected and colonized patients, contaminate hospital surfaces and have the ability to survive on surfaces for extended periods. Control of surface contamination can reduce the risk of cross-infection in hospitals, although the extent of the reduction remains controversial.

Conventional cleaning is not always effective for the elimination of pathogens. In a complex environment such as hospital wards, there are areas that are inaccessible and...
difficult to clean. In addition, sensitive medical equipment may not be compatible with liquid cleaning agents. Hydrogen peroxide is an effective sporicidal biocide, and several vapour/aerosol hydrogen-peroxide-based room disinfection technologies have emerged.\(^6,7,9\) These methods do not rely on the operator to ensure adequate distribution and repeatability, so have several potential advantages over conventional cleaning.\(^8\)

Two types of hydrogen-peroxide-based room disinfection system are available to the market in the UK. The Bioquell system (Clarus R, Bioquell, Andover, UK) vapourizes 30\% liquid hydrogen peroxide to generate hydrogen peroxide vapour (HPV), which is then delivered as a condensing vapour.\(^3,5\) The Sterinis system (SR2, Sterinis, now supplied as Glosair, Advanced Sterilization Products (ASP), Johnson & Johnson Medical Ltd, Wokingham, UK) creates an aerosol from a solution containing 5\% hydrogen peroxide and silver ions, called either ‘dry-mist hydrogen peroxide’ or ‘aerosolized hydrogen peroxide’ (aHP).\(^6,7,9–11\) No ‘head-to-head’ comparisons have been conducted to determine which system is more effective.\(^8\) Therefore, a comparison study was conducted to evaluate the efficiency, efficacy, and health and safety considerations of the two systems.

**Methods**

**Room description**

The test area consisted of two rooms to approximate a large hospital room with an ensuite bathroom, with 11 sampling points. The main room had an area of 50.1 m\(^3\) and the side room had an area of 13.2 m\(^3\) (Figure 1).

**Aerosolized hydrogen peroxide equipment**

The Sterinis equipment aerosolizes Sterusil, which contains a mixture of hydrogen peroxide (5\%), silver ions (<50 ppm) and orthophosphoric acid (<50 ppm).\(^6,7,9,10\) The Sterinis machine was placed in the corner of the room as instructed by the user manual. According to the manufacturer, the hydrogen peroxide aerosol has a particle size of approximately 8–12 \(\mu\)m and electrically charged particles adhere to particles in the air and on surfaces. The dose used was 6 mL/m\(^3\), as recommended by the manufacturer and used by other researchers.\(^7,9,10\)

**Hydrogen peroxide vapour equipment**

The Bioquell equipment generates HPV using 30\% liquid hydrogen peroxide, vapourized at 130 °C. The HPV decontamination equipment includes an HPV generator, an aeration unit (to break down the HPV catalytically after the exposure period) and an instrumentation module, which measures the concentration of hydrogen peroxide, temperature and relative humidity in the room. The instrumentation module monitors the conditions inside the room for both the HPV and aHP cycles. Equipment use was determined by the user manual. HPV was injected by a nozzle rotating in two directions until, according to the manufacturer, saturation of the air occurs (the dew point), at which point hydrogen peroxide begins to condense on to surfaces resulting in rapid killing of micro-organisms.\(^12\) The manufacturer recommends cycles of approximately 10 g/m\(^3\).

**Preparation of test discs for MRSA, C. difficile and A. baumannii**

Methods described by Otter and French to prepare test discs for MRSA NCTC 11939, A. baumannii NCTC 12156 and a C. difficile clinical isolate with a recoverable inoculum of approximately 10\(^9\) colony-forming units (cfu)/disc were used.\(^8\) Briefly, 500 \(\mu\)L of overnight culture of the vegetative bacteria in a brain heart infusion broth (Oxoid, Basingstoke, UK) or 300 \(\mu\)L of the spore suspension was centrifuged and resuspended in either water or bovine serum albumin (BSA; Sigma, Gillingham, UK) at 3\% or 10\% to simulate soiling. Sterile stainless steel discs (Apex Laboratories, Sanford, NC, USA) with a diameter of 10 mm were inoculated with 10 \(\mu\)L of the resulting suspension and air-dried overnight. To enumerate the surviving bacteria or spores, discs were transferred into 1 mL saline in individual plastic Bijou bottles, submerged for at least 15 min at room temperature, and sonicated at 60 Hz for 5 min for vegetative bacteria or 20 min for C. difficile. Five 10-fold serial dilutions of 10 \(\mu\)L into 90 \(\mu\)L saline were performed in a 96-well microtitre tray. The dilutions were plated on Columbia blood agar (CBA; Oxoid) for the vegetative bacteria and fastidious anaerobe agar (FAA; Oxoid) for C. difficile. To improve the limit of detection, the remaining 990 \(\mu\)L in the Bijou bottle was poured into a vial of tryptone soya broth (TSB; Oxoid) for vegetative bacteria or thioglycolate broth (bioMérieux, Basingstoke, UK) for C. difficile. The limit of detection of the serial dilutions was 100 cfu/test piece; therefore, growth in the broth in the absence of growth at the highest serial dilution was considered to represent <100 cfu/test piece. CBA plates, TSB and thioglycolate were incubated aerobically at 37 °C for 24 h. FAA plates were incubated anaerobically at 37 °C for 24 h.

![Figure 1. Room diagram and sampling positions. Positions 1–4 were in the corners of the main room in alternating floor and ceiling locations. Positions 5–8 were in the corners of the side room in alternating floor and ceiling locations. Position 8 was behind the door. Position 9 was underneath the bench in the centre of the room, out of direct line of sight of the generators. Positions 10 and 11 were on benches in the main room and the side room, respectively. Numbers in boxes indicate ceiling positions. BQ, location of Bioquell machine; ST, location of Sterinis machine.](image-url)
**G. stearothermophilus biological indicators**

*G. stearothermophilus* biological indicators (BIs) are a standard indicator of HPV decontamination. Tyvek paper-pouched BIs at a certified loading of >4 or >6-log spores/disc on stainless steel discs (Apex Laboratories, Sanford, NC, USA) were used. BIs were aseptically transferred to individual TSB vials and incubated at 57 °C for seven days. Turbidity indicated growth of the BI.

**Experimental summary**

To test the efficacy of the systems, test discs for MRSA, *A. baumannii* and *C. difficile*, suspended in water and then air-dried, were placed at each of the test locations in Figure 1. In addition, discs suspended in 3% and 10% BSA and then air-dried were placed at Positions 10 and 11 and exposed to aHP or HPV cycles. Following exposure, surviving bacteria or spores were enumerated, including three unexposed and dried control discs prepared in water, 3% and 10% BSA for each organism. Three cycles were conducted for each system. Four further cycles were conducted with each machine using 6- and 4-log *G. stearothermophilus* BIs. Due to concerns over the ability of the aHP to penetrate the Tyvek pouches of the BIs, pouched and unpouched 6- and 4-log BIs were used for the aHP cycles.

To test safety aspects of the systems, a hand-held hydrogen peroxide detector (Pac III, Draeger Blyth, UK) was placed outside the room to monitor any leakage. Readings were taken every 5 min. The Sterinis user manual does not recommend sealing around the door, whereas the Bioquell user manual recommends sealing the door with adhesive tape. Therefore, in one of the aHP cycles, the door of the room was not sealed and plastic sheeting was used to isolate the detector between the unsealed door and the operator outside the room. The gap between the floor and the door was approximately 5 mm.

To assess the efficiency of the systems, a comparison was made between the cycle times of the two systems by measuring the concentration of hydrogen peroxide using the calibrated hand-held sensor at three locations inside the room (centre of room, under table and in side room), 2 h after the start of the cycle. A 2-h delay was chosen as the recommended time to re-enter the room after the Sterinis cycle in accordance with the user manual. The Bioquell system recommends that the room should be re-entered once the measurable concentration of hydrogen peroxide has reached <1 ppm.

**Results**

The cycle dynamics of the aHP and HPV systems were considerably different (Figure 2). The aHP system resulted in a greater increase in humidity in the room, but achieved a considerably lower concentration of hydrogen peroxide (<50 vs >100 ppm for the aHP and HPV systems, respectively).

**Efficacy**

The HPV system inactivated more than 90% of the 6-log BIs and more than 95% of the 4-log BIs (Table I). In contrast, the aHP...
system inactivated <10% of the pouched 6-log BIs, <15% of the unpouched 6-log BIs, and approximately one-third of the 4-log BIs, regardless of whether they were pouched or unpouched.

The HPV system was generally more efficacious at inactivating the MRSA, _A. baumannii_ and _C. difficile_ test discs (Table II). The HPV system completely inactivated (>6-log reduction) MRSA dried from suspension in water from all replicates in nine of the 11 locations, _A. baumannii_ dried from suspension in water from all replicates in six of the 11 locations, and _C. difficile_ from all replicates in all locations, whether dried from a water or BSA-containing suspension. Most of the locations where _MRSA_ or _A. baumannii_ were not completely inactivated were only cultured by broth culture, which is indicative of low residual contamination. The addition of 3% and 10% BSA as simulated soiling reduced the efficacy of the HPV system on the _MRSA_ samples (although the system still generally achieved reductions in the range of 4–6 log), but not on the _A. baumannii_ or _C. difficile_ samples.

The aHP system did not completely inactivate _MRSA_ or _A. baumannii_ dried from suspension in water from all replicates at any of the locations (Table II). The system generally achieved 2–5-log reductions on _MRSA_ and 1–4-log reductions on _A. baumannii_ dried from suspension in water. The level of reduction achieved was dependent on location: inactivation in the side room was generally less than in the main room. Position 10, which was approximately 1.5 m away from the nozzle, gave a substantially higher decontamination level than the other locations. _MRSA_ and _A. baumannii_ samples suspended in 3% or 10% BSA proved difficult to inactivate for the aHP system, usually achieving a 1–3-log reduction. _C. difficile_ proved easier for the aHP system to inactivate than _MRSA_ or _A. baumannii_; _C. difficile_ was inactivated from all replicates from one location and was only detectable by broth culture in nine of the 11 locations. However, only a <1-log reduction was achieved at one of the locations, suggesting insufficient distribution of the aHP.

### Safety

The short-term exposure limit (STEL) for hydrogen peroxide is 2 ppm as a 15-min time weighted average. When the door of the room was not sealed with tape during an aHP cycle, the concentration of hydrogen peroxide exceeded 2 ppm for more than 2 h. Furthermore, the peak concentration could not be detected for 15 min because it was beyond the upper detection limit of the sensor (>20 ppm). When the door was sealed with adhesive tape, the concentration of hydrogen peroxide outside the room was <1 ppm for both systems.

### Efficiency

The mean hydrogen peroxide concentration at three points in the room, 2 h after the cycle started was 1.3 [standard deviation (SD) 0.4] ppm and 2.8 (SD 0.8) ppm for the four HPV and aHP cycles, respectively. The maximum reading was 1.7 ppm for HPV and 4.5 ppm for aHP. None of the readings were <2 ppm for the aHP cycles. This indicates that the room was not safe to enter at the manufacturer’s recommended time after the Sterinis cycles. HPV cycles were quicker due to a lower hydrogen peroxide level 2 h after the cycles started. One aHP cycle failed and was abandoned due to the hydrogen peroxide level being too low (peak 9.1 ppm).

### Discussion

Manual cleaning and disinfection is laborious and time consuming, does not always eradicate pathogens, its quality depends on the operator, and there is evidence that manual cleaning can spread bacteria on surfaces. Whole-room disinfection methods, such as the two hydrogen-peroxide-based methods investigated in this study, offer the potential for complete surface coverage, and remove the reliance on the operator for thorough and repeatable hospital room disinfection.

The in-vitro efficacy of the Bioquell HPV system has been demonstrated against a range of nosocomial pathogens, including _MRSA, A. baumannii, C. difficile_ and viruses. Several studies have demonstrated the superiority of the Bioquell system over conventional cleaning. HPV has been used for decontamination during outbreaks and routine use of HPV resulted in a significant reduction in _C. difficile_ infection in one US hospital.

Four studies have investigated the use of Sterinis aHP in healthcare applications. No studies have made a thorough investigation of the in-vitro efficacy of aHP against nosocomial pathogens, with the exception of _C. difficile_. Although all four healthcare studies reported substantial microbiological impact in some way, incomplete inactivation was a feature of the studies; for example, 13% of 146 _Bacillus_
<table>
<thead>
<tr>
<th>Organism</th>
<th>Control discs, mean log_{10} cfu/disc (SD)</th>
<th>Test discs, log_{10} cfu/disc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water 3% BSA 10% BSA</td>
<td>Loc 1</td>
</tr>
<tr>
<td>MRSA</td>
<td>7.2 (0.2) 7.6 (0.3) 7.8 (0.0)</td>
<td>HPV cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPV cycle 2</td>
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<tr>
<td></td>
<td></td>
<td>HPV cycle 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Median log)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>6.0 (1.7) 6.4 (2.1) 6.9 (1.7)</td>
<td>aHP cycle 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aHP cycle 2</td>
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<tr>
<td></td>
<td></td>
<td>aHP cycle 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Median log)</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>5.2 (0.7) 6.1 (0.5) 6.2 (0.8)</td>
<td>HPV cycle 1</td>
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<tr>
<td></td>
<td></td>
<td>HPV cycle 2</td>
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<td>HPV cycle 3</td>
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<td>(Median log)</td>
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BSA, bovine serum albumin; SD, standard deviation; cfu, colony-forming unit.
Locations where no bacteria or spores were cultured are marked in bold. <2.0 refers to samples where growth was only identified in the broth culture, representing <100 cfu.
atropheus BIs remained viable after exposure; one of five chair arms remained contaminated with MRSA, and MRSA remained viable in the home environment after exposure; and C. difficile was cultured from five of 10 (50%) rooms after exposure in one study and three of 15 (20%) rooms in another study. No published data are available on the clinical impact of aHP systems in the healthcare setting.

In this study, the HPV system inactivated most of the BIs, but the aHP system was not effective for the inactivation of 4- or 6-log BIs, whether pouched or unpouched, which is consistent with the findings of others. The log reductions for the HPV system were consistently greater than for the aHP system; this effect was more evident for the catalase-positive MRSA and A. baumannii than for the metabolically inert C. difficile spores. The relative resistance of catalase-positive bacteria to HPV has been noted previously. Better germicidal results were observed at the locations near the aHP machine, and poorer results were noted in the side room. In contrast, the HPV system demonstrated a more even distribution. The heterogeneous distribution of aHP is most likely explained by the limited diffusion of aerosol, corner location and unidirectional injection, compared with the vapour-phase, central location and multi-directional injection of the HPV system. Taken together, these data suggest that the aHP system is considerably less efficacious than the HPV system.

The germicidal activity of aHP and, to a lesser extent, HPV varied considerably between repeat cycles. This could be due to variation in the concentration of contamination on the in-house prepared discs, changes in the starting temperature and relative humidity, or variation in equipment performance.

The concentration of hydrogen peroxide inside the room during the cycles for both aHP and HPV systems exceeds the STEL, so procedures must be in place to ensure that no personnel are exposed. Based on the study findings, it is recommended that doors and air vents should be sealed with adhesive tape for both systems. The HPV system achieved a higher concentration of hydrogen peroxide in the room, and the system provides several safety advantages, including routine monitoring to detect leakage of hydrogen peroxide (which is odourless), remote control of the generator, active aeration, and real-time monitoring of environmental parameters inside the room, which would detect deviations from a normal cycle.

The results indicate that the room was not safe to enter at the time recommended by the Sterinis user manual. The sensors used have been designed for the detection of HPV, although both the hand-held and in-room detectors are electrochemical hydrogen peroxide cells which are unlikely to underestimate true readings. The size of the gap between the floor and the door was approximately 5 mm. More leakage would be expected for doors with a larger gap and doors with vents. One aHP cycle failed and was abandoned as the level of hydrogen peroxide was too low. Two other studies of aHP systems have reported technical failures of some kind.

This study has several limitations. Only a small number of pathogens were included, and only one strain per pathogen; the impact of different cycle parameters for both systems was not investigated; and actual surface contamination was not measured. The log loadings used in this study are higher than those typically encountered on hospital surfaces, but were chosen to represent a worst-case challenge. Future evaluation of the efficacy of these systems with lower log loadings would be useful. The Bioquell system is more expensive than the Sterinis system, but the authors do not have any specific costing data. Despite these limitations, based on the study data, the HPV system was safer to operate, slightly faster and achieved a greater level of biological inactivation than the aHP system. Further studies of the microbiological and clinical impact of these and other room disinfection methods are warranted.

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Conflicts of interest statement

None declared.

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References


