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ORIGINAL ARTICLE

Evaluating different concentrations of hydrogen peroxide in an automated room disinfection system

L.E. Murdoch, L. Bailey, E. Banham, F. Watson, N.M.T. Adams and J. Chewins

Wickham Laboratories, Gosport, Hampshire, UK

Significance and Impact of the Study: This research allows hospital infection control teams to consider the impact and risks of using low concentrations of hydrogen peroxide for disinfection within their facilities, and to question automated room disinfection system providers on the efficacy claims they make. The evidence that low concentration hydrogen peroxide solutions do not rapidly, autonomously break down, is in contradiction to the claims made by some hydrogen peroxide equipment providers and raises serious health and safety concerns. Facilities using hydrogen peroxide systems that claim autonomous break down of hydrogen peroxide should introduce monitoring procedures to ensure rooms are safe for re-entry and patient occupation.

Keywords
biological indicator, concentration, decontamination, exposure limit, hydrogen peroxide, methicillin resistant *Staphylococcus aureus*.

Correspondence
Lynne Murdoch, Wickham Laboratories Limited, Hoeford Point, Gosport, Hampshire PO13 0AU, UK.
E-mail: lynnem@wickhamlabs.co.uk

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Abstract

A comparative study was made on the efficacy of 5, 10 and 35% weight by weight (w/w) hydrogen peroxide solutions when applied using an automated room disinfection system. Six-log biological indicators of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Geobacillus stearothermophilus* were produced on stainless steel coupons and placed within a large, sealed, environmentally controlled enclosure. Five percent hydrogen peroxide was distributed throughout the enclosure using a Bioquell hydrogen peroxide vapour generator (BQ-50) for 40 min and left to reside for a further 200 min. Biological indicators were removed at 10-min intervals throughout the first 120 min of the process. The experiment was repeated for 10 and 35% hydrogen peroxide solutions. Five percent and 10% hydrogen peroxide solutions failed to achieve any reduction of MRSA, but achieved full kill of *G. stearothermophilus* spores at 70 and 40 min respectively. Thirty-five percent hydrogen peroxide achieved a 6-log reduction of MRSA after 30 min and full kill of *G. stearothermophilus* at 20 min. The concentration of 5% hydrogen peroxide within the enclosure after the 200-min dwell was measured at 9·0 ppm. This level exceeds the 15-min Short Term Exposure Limit (STEL) for hydrogen peroxide of 2·0 ppm. Users of automated hydrogen peroxide disinfection systems should review system efficacy and room re-entry protocols in light of these results.

Introduction

The use of hydrogen peroxide-based automated room disinfection systems in pharmaceutical and health care environments is becoming increasingly common, due to its ease of use and the expanding body of scientific literature supporting its efficacy. The bulk of the scientific literature has been produced through the evaluation of 30–35% concentrations of hydrogen peroxide delivered as a vapour. Some literature exists evaluating the efficacy of 5% concentrations of hydrogen peroxide, along with direct head-to-head efficacy studies between 5 and 35% solutions (Andersen *et al.* 2006, 2010; Pitten *et al.* 2008; Holmdahl *et al.* 2011; Piskin *et al.* 2011; Fu *et al.* 2012). These head-to-head studies show 30–35% peroxide to provide superior micro-organism inactivation compared to 5% peroxide. The studies were designed to evaluate the hydrogen peroxide concentration in combination with the

manufacturer's specific application device, thus introducing the potential variable of the application mechanism to the efficacy result. No European efficacy performance standard currently exists for automated room disinfection systems (although a European task force is currently developing an airborne disinfection standard based on the French National Standard NF T 72-281:2014), making it difficult for health care professionals to confirm a manufacturer's efficacy claims and directly compare different technologies and disinfectants.

Hydrogen peroxide is an oxidizing agent. It is nonselective, making it difficult for microbiological organisms to generate resistance to it, although the enzyme catalase produced by some micro-organisms can break down hydrogen peroxide. However, its nonselective nature makes it potentially hazardous to humans, primarily through an inhalation exposure route. The long-term (8 h) and short-term (15 min) occupational exposure limits for hydrogen peroxide are 1·0 and 2·0 ppm respectively. Ernstgard *et al.* (2012) showed nasal and throat irritation in healthy volunteers when exposed to 2·2 ppm for 2 h. The US CDC (2016) lists upper airway irritation, inflammation of the nose, hoarseness, shortness of breath and tightening of the chest as possible reactions to inhalation of low levels of hydrogen peroxide. Children may be more vulnerable to reactions due to the smaller diameter of their airways. Some manufacturers of hydrogen peroxide room disinfection systems stipulate the use of catalytic aeration or 'scrubbing' units to break down the hydrogen peroxide once the disinfection process has taken place. Others claim that the hydrogen peroxide autonomously breaks down, with the room available for re-entry within a matter of hours.

The aim of this study was to clarify the performance efficacy of hydrogen peroxide solution through the application of three different concentrations, 5, 10 and 35% w/w, to a standardized challenge using the same application equipment in a controlled enclosure and to monitor the residual hydrogen peroxide levels within the enclosure post disinfection.

Results and discussion

Thirty-five percent hydrogen peroxide showed greater efficacy and speed of kill than 10% hydrogen peroxide, which in turn showed a greater speed of kill than 5% peroxide (Figs 1 and 2). For *Geobacillus stearothermophilus*, the ability to achieve full kill over a period of 60 min using 35% hydrogen peroxide compared to 5% hydrogen peroxide was statistically significant ($P = 0{\cdot}005$), but was not significant compared to 10% peroxide ($P = 0{\cdot}28$). The ability of 10% peroxide to achieve full kill over the same period compared to 5% peroxide was also significant ($P = 0{\cdot}05$). For MRSA, the reductions achieved by 35% peroxide were statistically significant compared to both 5% ($P < 0{\cdot}0001$) and 10% peroxide ($P < 0{\cdot}0001$). Ten percent peroxide also produced a statistically significant reduction compared to 5% peroxide ($P = 0{\cdot}002$). The presence of soiling (0·3% BSA) rendered the 5 and 10% solutions ineffective against a 6-log loading of MRSA and produced variable kill with 35% peroxide between 30 and 100 min. These results correspond to the work of Fu *et al.* (2012), who identified reduced performance of 5% peroxide in the presence of soiling. The commercially available *G. stearothermophilus* biological indicators used, however, are manufactured to be free of soil and show a clear relationship between peroxide concentration and microbiological kill. The results show the importance of removing gross soil from surfaces prior to conducting hydrogen peroxide-based room decontamination; and, the

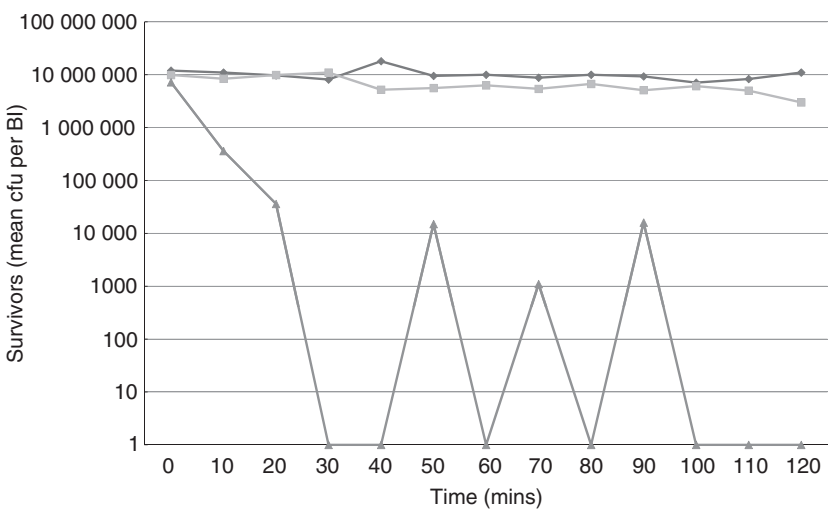

Figure 1 Survivor curve of MRSA exposed to 5, 10 and 35% hydrogen peroxide vapour. (—◆—) 5%, (—■—) 10% (—▲—) 35%.

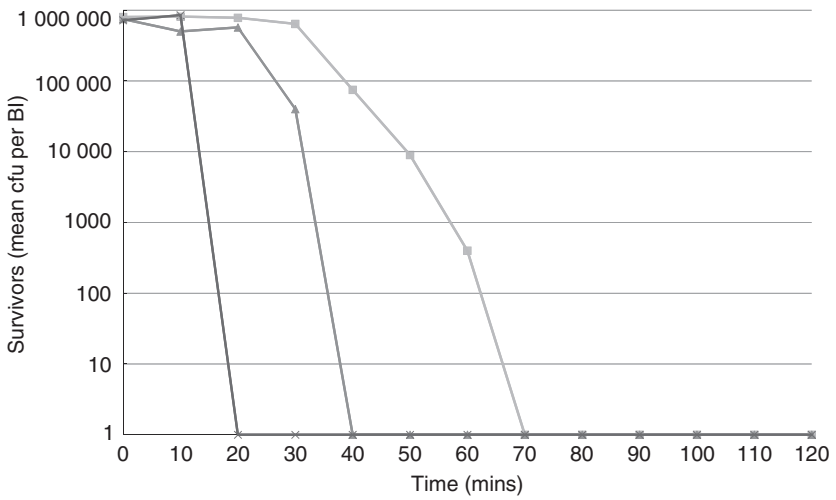

Figure 2 Survivor curve of *Geobacillus stearothermophilus* exposed to 5, 10 and 35% hydrogen peroxide vapour. (—■—) 5%, (—▲—) 10% (—×—) 35%.

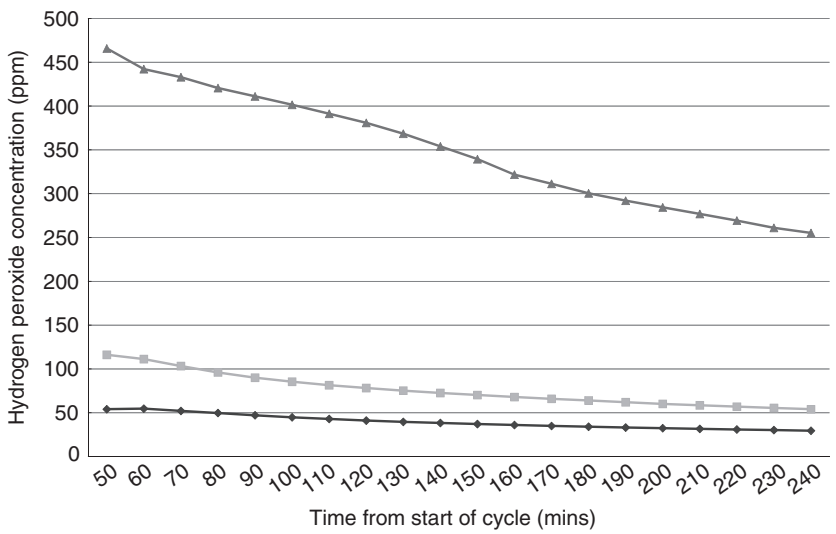

Figure 3 Hydrogen peroxide autonomous breakdown and concentration over time post peroxide introduction (40 min). (—◆—) 5%, (—■—) 10% (—▲—) 35%.

significantly increased contact time required by 5 and 10% hydrogen peroxides to achieve an equivalent kill to 35% hydrogen peroxide solution on a pure, soiling free presentation. MRSA is a catalase-producing organism and the presence of catalase has been shown to reduce the efficacy of hydrogen peroxide (Otter and French 2009). Catalase will likely have contributed to the lack of MRSA inactivation by the 5 and 10% hydrogen peroxide solutions. Users of 5% hydrogen peroxide room decontamination systems should review the duration and contact times of their decontamination cycles in the light of this evidence. Users should not accept efficacy data generated using 30–35% hydrogen peroxide solutions as evidence to support the performance of 5 or 10% hydrogen peroxide solutions.

Hydrogen peroxide autonomously breaks down within an enclosure; however, the rate of decay slows as the concentration decreases (Fig. 3). Even with the lowest concentration 5% peroxide, the enclosure remained at a concentration of 9·0 ppm after >3 h. This is significantly above the 1·0 ppm occupational exposure limit where patients could be safely readmitted to a room. These results show that patients remain at risk of above STEL hydrogen peroxide exposure where natural breakdown of peroxide is encouraged by the manufacturer. Forced aeration using aeration units and monitoring of the hydrogen peroxide concentration level in the room is paramount for ensuring patient safety upon re-entry.

Materials and methods

Test solutions were prepared by diluting stock 35% w/w hydrogen peroxide solution with sterile distilled water.

Solutions were titrated using potassium iodide to confirm precise concentrations.

A Bioquell BQ-50 hydrogen peroxide vapour (HPV) generator was placed in the centre of an 80 m^3 test enclosure along with a calibrated hydrogen peroxide monitoring module (Bioquell UK, Andover). The enclosure was fitted with glove and transfer ports to enable manipulation and removal of the biological indicators. The test enclosure was controlled to 22 ± 2°C and 45 ± 5% RH for the start of each gassing experiment.

Staphylococcus aureus (MRSA) NCTC 11939 was subcultured onto Tryptone Soya Agar (TSA) (Oxoid, Basingstoke, UK) and incubated at 30–35°C for 18–24 h. Cultures were harvested in Peptone in Saline (PES) and adjusted to $1{\cdot}0 \times 10^8$ CFU ml^{-1} (colony forming units) using a spectrophotometer. The suspension was centrifuged at 3260 ***g*** for 15 min to form a pellet. The supernatant was aspirated off and replaced with 0·3% (0·3 g of Bovine albumin in 100 ml of sterile deionized water) Bovine Serum Albumin (BSA) at the same volume aspirated (e.g. 10 ml PES removed, 10 ml BSA replaced). About 0·3% BSA was used, as this is a recognized presentation in European disinfectant testing standards. Plate counts were performed in triplicate to confirm the inoculum level. Sterile, clean stainless steel discs, 10 mm in diameter were laid out on a sterile field in a laminar flow cabinet. Discs were inoculated with 20 μl of the prepared suspension using an automatic pipette, to give an inoculum of approx. $1{\cdot}0 \times 10^6$ CFU per BI. Test discs were left to dry thoroughly for 1·5 h in a laminar flow cabinet with the fans switched off. Thirteen test discs were placed into a 140-mm Petri dish and transferred into the test enclosure. One test disc was held in the laboratory as a control.

Geobacillus stearothermophilus (ATCC 12980) Tyvek pouched biological indicators (BIs) with a $1{\cdot}0 \times 10^6$ certified population of spores were commercially procured (Bioquell UK). Thirteen indicators were transferred into the test enclosure and one indicator remained in the laboratory as a control.

Sterile 30-ml containers containing 10 ml deionized water with 0·1% Tween 80 (DEWT 0·1%) were labelled and transferred to the test room.

The BQ-50 was loaded with the appropriate concentration hydrogen peroxide solution and the following cycle was conducted for each experiment. Around 640 g of solution was injected over a period of 40 min, followed by a 200-min dwell period. A single biological indicator for each test organism was placed into the appropriately labelled 30-ml container and removed via the transfer port for enumeration. This sampling procedure was repeated every 10 min for 120 min.

Monitoring of the enclosure was carried out for a further 120 min using the hydrogen peroxide-monitoring module inside of the room and a calibrated low concentration hydrogen peroxide Draeger X-AM 5100 monitor (Draeger, Blyth, UK). The Draeger X-AM 5100 was introduced into the enclosure at 30-min intervals, via the transfer port.

Positive and negative controls were implemented and a method suitability study carried out to validate the method and results. Positive controls consisted of tokens removed at time zero and enumerated. Negative controls consisted of sterile diluent plated out and incubated.

Upon removal from the enclosure, BIs were allowed to soak for 15 min at ambient temperature and then sonicated at 60 Hz for 5 min. Following sonication, 1 ml was removed and placed into 9 ml of deionized water. Further serial dilutions were performed to 10^{-5} dilution. Duplicate 1-ml aliquots of all dilutions (10^{-1} to 10^{-5}) were plated into 90-mm Petri dishes and poured with TSA.

MRSA plates were incubated at 30–35°C for 48–72 h. *Geobacillus stearothermophilus* plates were incubated at 55–60°C for 48–72 h. After incubation the colonies were counted on each plate and presented as an average CFU per biological indicator.

Statistical analysis was conducted using a Chi squared analysis of kill *vs* growth for experimentation using *G. stearothermophilus*. A one-way ANOVA was applied to MRSA enumerations over the exposure period. The statistical analysis is limited due to the small data set available.

Conflicts of Interest

Dr L.E. Murdoch and L. Bailey have no conflicts of interest to declare. E. Banham, F. Watson, N.M.T. Adams and J. Chewins are employees of Bioquell UK a provider of hydrogen peroxide decontamination systems.

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