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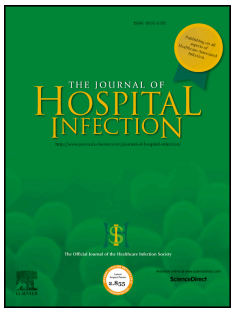

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PII: S0195-6701(16)00083-9

DOI: 10.1016/j.jhin.2016.01.016

Reference: YJHIN 4738

To appear in: *Journal of Hospital Infection*

Received Date: 9 July 2015

Accepted Date: 12 January 2016

Please cite this article as: Ali S, Muzslay M, Bruce M, Jeanes A, Moore G, Wilson APR, Efficacy of two hydrogen peroxide vapour aerial decontamination systems for enhanced disinfection of meticillin-resistant *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Clostridium difficile* in single isolation rooms, *Journal of Hospital Infection* (2016), doi: 10.1016/j.jhin.2016.01.016.

Efficacy of two hydrogen peroxide vapour aerial decontamination systems for enhanced disinfection of meticillin-resistant *Staphylococcus aureus, Klebsiella pneumoniae* and *Clostridium difficile* in single isolation rooms

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SUMMARY

Background: Hydrogen peroxide vapour (HPV) disinfection systems are being used to reduce patients' exposure to hospital pathogens in the environment. HPV whole-room aerial disinfection systems may vary in terms of operating concentration and mode of delivery.

Aim: To assess the efficacy of two HPV systems (HPS1 and HPS2) for whole-room aerial disinfection of single isolation rooms (SIRs).

Methods: Ten SIRs were selected for manual terminal disinfection after patient discharge. Test coupons seeded with biological indicator (BI) organisms [~10^6 colony-forming units (cfu) of meticillin-resistant *Staphylococcus aureus* (MRSA) or *Klebsiella pneumoniae*, or ~10^5 cfu *Clostridium difficile* 027 spores] prepared in a soil challenge were placed at five locations per room. For each cycle, 22 high-frequency-touch surfaces in SIRs were sampled with contact plates (~25 cm^2) before and after HPV decontamination, and BIs were assayed for the persistence of pathogens.

Findings: Approximately 95% of 214 sites were contaminated with bacteria after manual terminal disinfection, with high numbers present on the SIR floor (238.0–352.5 cfu), bed control panel (24.0–33.5 cfu), and nurse call button (21.5–7.0 cfu). Enhanced disinfection using HPV reduced surface contamination to low levels: HPS1 [0.25 cfu, interquartile range (IQR) 0–1.13] and HPS2 (0.5 cfu, IQR 0–2.0). Both systems demonstrated similar turnaround times (~2–2.5 h), and no differences were observed in the efficacy of the two systems against BIs (*C. difficile* ~5.1 $\log_{10}$ reduction; MRSA/*K. pneumoniae* ~6.3 $\log_{10}$ reduction). Despite different operating concentrations of hydrogen peroxide, MRSA persisted on 27% of coupons after HPV decontamination.

Conclusion: Enhanced disinfection with HPV reduces surface contamination left by manual terminal cleaning, minimizing the risks of cross-contamination. The starting concentration and mode of delivery of hydrogen peroxide may not improve the efficacy of decontamination

in practice, and therefore the choice of HPV system may be based upon other considerations such as cost, convenience and logistics.

Keywords:

Hydrogen peroxide

Whole-room aerial decontamination

Infection control

Clostridium difficile

MRSA

Klebsiella pneumoniae

<A>Introduction

Despite recent improvements in the UK, reducing healthcare-acquired infections remains a priority for many hospitals. Control measures have focused on the hands of staff as a major vector of transmission. However, the standard of cleaning of the environment is at least as important in terms of both direct transmission to the patient, and transmission to staff hands and subsequently to the patient.[1] A previous study showed that enhanced cleaning not only reduced the bacterial load in the environment, but also reduced the number of organisms on staff hands.[2]

High-frequency-touch surfaces in near-patient areas may become contaminated rapidly with organisms disseminated by a colonized patient occupying that room,[3] and may remain contaminated for extended periods of time.[4–6] Contaminating organisms may be picked up on the hands of patients, healthcare staff and visitors, and spread to surfaces beyond the patient isolation room.[7–10]

Although bed areas in isolation rooms are decontaminated upon discharge of the patient, terminal disinfection is not fully effective and some organisms remain. Patients are at greater risk of acquisition if a previous occupant was colonized with meticillin-resistant *Staphylococcus aureus* (MRSA) or other hospital pathogens,[11] while asymptomatic carriers may not be recognized as a clinical risk and represent a major source of surface contamination. The importance of asymptomatic carriers of *Clostridium difficile* from the community in hospital spread has been demonstrated recently, and suggests that the role of the environment may be important in controlling acquisition.[12] Furthermore, bacteria and spores can be spread to uncontaminated surfaces during manual cleaning and areas in the patient environment frequently missed by cleaning personnel.[13,14] The control of the spread of hospital pathogens requires an effective decontamination procedure for surfaces in the clinical environment.

Hydrogen peroxide is a highly active biocide that exhibits activity through the generation of hydroxyl free radicals that penetrate the cell wall to attack lipids, proteins and DNA.[15] It is active against viruses, spores and fungi as well as bacteria.[16] Although toxic when used as a vapour, it breaks down to water and oxygen alone.

Hydrogen peroxide decontamination systems are widely used in an attempt to overcome deficiencies in cleaning, and to reduce the risk of acquisition by the patient, particularly of *C. difficile.*[17,18] This study compared two widely available aerial whole-room hydrogen peroxide decontamination systems. The efficacy of both systems was assessed against in-house biological indicator (BI) coupons seeded with high concentrations (~10^5–10^6) of contaminating organisms (*C. difficile* 027 spores, *Klebsiella pneumoniae* or MRSA), and challenged with either low-level soiling or heavy soil simulating biological fluid or faecal material. Reduction of surface contamination in the patient room was assessed to determine the efficacy of each system in disinfecting surfaces that are difficult to clean or frequently missed.

<A>**Methods**

<B>Selection and preparation of test rooms

Ten single isolation rooms at a London teaching hospital were selected for enhanced disinfection using one of two hydrogen peroxide vapour (HPV) decontamination systems (HPS1 and HPS2) upon discharge of the occupying patient. A manual terminal clean [manual wiping of surfaces using 1000 ppm solutions of peracetic acid disinfectant (DiffX, MTP Innovations, Huddersfield, UK)] of the room was performed by the facilities management team as stipulated by hospital protocol. Rooms selected were of similar dimension (approximately 4 m x 5.5 m x 2.7 m) and included an en-suite bathroom. During the course of the trial, each test room was selected for decontamination once.

<B>Decontamination systems

<C>HPS1 (Bioquell Q10, Bioquell, Andover, UK)

HPS1 uses hydrogen peroxide solution at 30% which is heated to 130°C to produce vapour of the desired particle size. The generator is accompanied by an aeration unit to catalyse the degradation of hydrogen peroxide, and an oscillating fan was placed in the doorway of the bathroom to facilitate the circulation of hydrogen peroxide in the room.

<C>HPS2 (Deprox, Hygiene Solutions, Kings Lynn, UK)

HPS2 generates HPV with 4.9% hydrogen peroxide solution using piezo-ultrasonics. At the end of each decontamination cycle, HPV is degraded with an aeration unit integrated into the generator unit. No further equipment is required to facilitate air flow during the decontamination and degradation cycles.

<B>Operation of the hydrogen peroxide vapour decontamination unit

Decontamination systems were operated following the manufacturers' recommendations. Each system was placed in the centre of the test room, all external doors were sealed using insulation tape, and ventilation ducts were sealed using an airtight cover (provided by each manufacturer). HPV decontamination cycle programmes were initiated remotely from outside the room. Hand-held sensors were placed outside the external doors to monitor the leakage of hydrogen peroxide during the decontamination cycle. The HPS1 unit was operated by a trained engineer (Bioquell), while the HPS2 module was operated by hospital staff following training by a dedicated member of the issuing manufacturer (i.e. Hygiene Solutions). The generator module recorded the concentration of hydrogen peroxide produced, room temperature and relative humidity during the decontamination cycle.

<B>Preparation of in-house biological indicators

Three in-house BIs were prepared from clinical isolates: MRSA (EMRSA-15 variant B1), an extended-spectrum beta-lactamase-producing *K. pneumoniae* and *C. difficile* (polymerase chain reaction 027 spores; clinical isolate).

Sterile nutrient broth (10 mL) (Oxoid, Basingstoke, UK) inoculated with MRSA or *K. pneumoniae* was mixed thoroughly and incubated aerobically at 37°C for 18 h. Broth cultures were centrifuged at 3000 rpm (~1500 ***g***; Jouan CR3i centrifuge, Thermo, Basingstoke, UK) for 10 min, and the remaining pellet was resuspended in 10 mL of sterile bovine serum albumin (BSA; Sigma-Aldrich, Gillingham, UK) at a concentration of 0.03% (w/v) or 10% (w/v) representing low and heavy soil loads, respectively.

Spore suspensions of *C. difficile* were prepared and stored using methods described previously.[19] The presence of spores (phase-bright cells) was confirmed by phase-contrast microscopy, and the suspension was determined to be free (~95%) of organic debris. Spore titres of stock *C. difficile* spore suspensions were confirmed by plate culture and adjusted (by combining several spore preparations) to produce 10-mL aliquots with an inoculum titre of approximately 10^6 colony-forming units (cfu)/mL. Before each experiment, *C. difficile* stock spore suspensions were concentrated by centrifugation at 12,000 rpm (13,800 ***g***; Heraeus Fresco microcentrifuge, Thermo, Basingstoke, UK) for 1 min and resuspended in 1 mL of sterile BSA at 0.03% (w/v) or 1 mL of synthetic body fluid[20] [5% (w/v) tryptone, 5% (w/v) BSA, 0.4% (w/v) mucin (from porcine stomach, Type II) prepared in sterile phosphate-buffered saline (PBS)].

All soil suspensions were filter sterilized by passing through a nitrocellulose membrane filter (0.45 µm pore size; VWR International, Leighton Buzzard, UK) using a syringe, and refrigerated (2-5°C) in sterile universal tubes prior to use.

<B>*Preparation of stainless steel coupons*

Stainless steel coupons (1 cm x 1 cm, grade 304) were soaked in disinfectant solution (1000 ppm; Actichlor, EcoLab Ltd, Swindon, UK) for 1 h, rinsed three times in sterile de-ionized water, then immersed in 70% ethanol solution for 5 min before allowing to air-dry. The coupons were autoclaved (121°C for 15 min) and stored in sterile containers until required.

<B>*Inoculation of stainless steel carrier coupons with biological indicators in microplate arrays*

A sterile stainless steel coupon was placed aseptically in an array on the inside of an upturned lid of an inverted 12-well microplate (sterile grade; well diameter ~1.5 cm; Corning CellBIND, Sigma-Aldrich). Coupons were inoculated with 10 µL of bacterial (~10^6 cfu) or spore (~10^5 cfu) suspension prepared in appropriate test soil. The inoculum was spread over the 1-cm^2 area using a pipettor tip, and then covered by placing the microplate base (inverted) on to the lid. Microplates were sealed using Parafilm M tape (Sigma-Aldrich) for transport.

<B>*Evaluation of efficacy of hydrogen peroxide decontamination systems*

Inverted microplates, each containing test coupons (*N*=3), were placed at five pre-determined sites (Figure 1). Immediately before initiating the HPV system, the microplates were opened (i.e. the inverted base of the microplate was removed), exposing the test coupons to the hydrogen peroxide. Each exposed microplate array was duplicated with an identical control array that remained sealed throughout the HPV decontamination cycle. A hydrogen peroxide indicator strip was placed in all control microplate arrays to detect leakage of hydrogen peroxide into the microplate cavity.

Upon completion of the decontamination cycle, hydrogen peroxide in the room was purged to a safe level (>1 ppm; monitored by the hydrogen peroxide control unit) before the

test room was deemed safe to enter. All exposed (test) microplates were resealed and returned to the laboratory (within 1–2 h) with the control arrays. Coupons were returned to the laboratory and transferred aseptically to a universal tube containing either 10 mL (for non-exposed, control coupons) or 1 mL (for exposed, test coupons) of neutralizing solution [sodium thiosulphate 0.1% w/v, Tween 80 3.0% w/v, lecithin 0.3% w/v (Sigma-Aldrich) prepared in sterile PBS]. Five sterile (autoclaved) glass beads (~4 mm diameter) were added to each universal tube, and the sample was incubated at room temperature for 10 min before being vortexed for 1 min. The resulting suspension was diluted 100-fold and 0.1 mL (control) or 0.5 mL (test) aliquots of each dilution were plated on to blood agar (Oxoid) or agar specific for *C. difficile* (Brazier's agar; Oxoid). All plates were incubated under appropriate atmospheric conditions at 37^{o}C for 24–48 h.

<B>Validation of neutralizer efficacy against hydrogen peroxide disinfection

Non-inoculated (blank) stainless steel coupons were exposed (in triplicate) to a hydrogen peroxide cycle. After treatment, exposed (blank) coupons and non-exposed (control) coupons were inoculated with 10-µL aliquots of BI (~10^3 cfu) organisms suspended in corresponding soil suspensions as above, and incubated at room temperature for 60 min. Each coupon was transferred to a universal tube containing 1 mL of neutralizing solution as above. Suspensions were serially diluted and plated. Numbers of BI organisms recovered from exposed and control coupons showed no significant difference, demonstrating that the neutralizing solution was non-toxic and effective at quenching residual bactericidal activity.

<B>Sampling of environmental surfaces

Twenty-two surfaces in each test room (Table I) that represented high-frequency-touch sites and surfaces that were difficult to access for manual cleaning were sampled using tryptone

soya agar contact plates (PRO-TECT; diameter 55 mm; Oxoid), pre-supplemented with neutralizing agents to inactivate antimicrobial activity of residual cleaning products on test surfaces. Each surface was sampled before exposure to hydrogen peroxide and immediately after the HPV decontamination cycle. Contact plates were transported to the laboratory (within 1–2 h) and incubated at 37°C for 48 h prior to reading.

<B>Statistics

Medians for test or control group(s) were calculated, and differences between sample populations were determined using the Mann–Whitney *U*-test. The effect of sampling location upon numbers present on BI coupons was evaluated using the Kruskal-Wallis (χ^2) test. The level of significance was set at α=0.05.

<A>Results

In total, 10 rooms were selected for HPV treatment (*C. difficile:* 10 rooms; MRSA: five rooms; *K. pneumoniae:* five rooms). No colour changes (from white to green/blue) were observed on indicator strips, indicating that control microplates were sealed adequately during HPV disinfection cycles.

Coupons were placed at Sites 1–5 (Table I) in rooms prepared for HPS1 or HPS2 disinfection. Approximately 5.7 $\log_{10}$ (±0.72) cfu *C. difficile* spores, 6.3 $\log_{10}$ (±0.40) cfu MRSA and 6.5 $\log_{10}$ cfu (±0.80) cfu of *K. pneumoniae* in soil suspension were recovered from non-exposed (control) coupons (Table II). Exposure to hydrogen peroxide achieved a 5.1 $\log_{10}$ reduction in *C. difficile* spores; there was no difference in the efficacy achieved by either decontamination system regardless of soiling or position (*P*>0.05). Similarly, MRSA and *K. pneumoniae* were reduced by approximately 6.3 $\log_{10}$ cfu in all areas of the test room.

<insert Tables I and II near here>

Environmental surface samples were taken from 22 sites in each side-room after terminal (manual) cleaning (i.e. before HPV decontamination) and after HPV treatment. After terminal cleaning, 96% (414/431) of the surfaces sampled were contaminated with bacteria. Similar numbers of bacteria were present on surfaces in side-rooms prepared for decontamination with HPS1 (21.0 cfu/25cm^2, IQR 7.5–45.0, *N*=217) or HPS2 (28.0 cfu/25 cm^2, IQR 7–67.5, *N*=214) (*P*>0.05).

The floor area behind the entrance door to side-rooms remained most highly contaminated after terminal cleaning (Sample Point 13; Table II), with high numbers also present on the bed control panel, nurse call button, chair seat, toilet flush and bathroom floor.

Both systems were effective for the decontamination of surfaces and had turnaround times of 2–2.5 h (Table II). Aerobic colony counts recovered from 109/217 surfaces (50.2%) exposed to HPS1 and from 106/214 surfaces (49.5%) exposed to HPS2 fell to below the detection limit (0 cfu/25cm^2). There was no significant difference in the numbers of bacteria remaining on surfaces following exposure to HPS1 (0.25 cfu/25cm^2, IQR 0–1.13, *N*=108) or HPS2 (0.5 cfu/25cm^2, IQR 0–2.0, *N*=108) (*P*>0.05).

The outside door handles to the isolation rooms (Table II, Site 13) remained contaminated with bacteria before HPV treatment (HPS1: 14.5 cfu, IQR 7.25–25.3; HPS2: 26.0 cfu, IQR 16.3–56.8) and after HPV treatment (HPS1: 9.5 cfu, IQR 5.8–20.8; HPS2: 5.0 cfu, IQR 1.5–17.8).

<A>**Discussion**

Terminal cleaning of a room is intended to prevent patient-to-patient cross-infection by reducing/eradicating pathogens on surfaces to numbers that make transmission unlikely. The

reduction in bacterial load was variable, such that transmission was still a risk from high-frequency-touch sites. Using biological indicators, both hydrogen peroxide systems achieved >5 $\log_{10}$ reduction in spores of *C. difficile* and 6 $\log_{10}$ reduction of MRSA and *K. pneumoniae*, even in the presence of heavy soiling. This study suggests that the choice between the systems can be determined by cost and convenience rather than efficacy.

Hydrogen peroxide aerial decontamination systems have been shown to be effective in reducing environmental contamination and consequent acquisition of infection.[21] The prevalence of *C. difficile* may be reduced when hydrogen peroxide is used.[22] During an outbreak of *C. difficile* infection, hydrogen peroxide decontamination was associated with a reduction of environmental isolation of the organism from 11/43 (25%) to 0/37 (0%) cultures.[17] A retrospective analysis of 334 rooms vacated by *C. difficile* patients and decontaminated using hydrogen peroxide or hypochlorite demonstrated that, compared with standard cleaning, hydrogen peroxide decontamination reduced the rate of acquired *C. difficile* (rate ratio 0.65, 95% confidence interval 0.50–0.79).[21]

In an attempt to to reduce the incidence of hospital infections, hydrogen peroxide systems may be implemented to overcome potential problems associated with operator use, such as varied distribution of surfaces cleaned, and the contact time of cleaning agents on surfaces during manual terminal disinfection. Further, inaccessible or difficult-to-clean surfaces may be missed or cleaned inadequately using conventional manual cleaning. Aerial whole-room disinfection using HPV in addition to conventional terminal cleaning was found to be highly effective in reducing the level of aerobic bacterial contamination on all sites sampled.

Although both HPV systems demonstrated >5 $\log_{10}$ reductions of the test bacteria when inoculated on to BI coupons, between 2 and 816 cfu MRSA remained on some coupons (Table III). These findings suggest that efforts to further reduce cleaning turnover by

substituting manual terminal cleaning of the isolation room with automated whole-room disinfection using HPV is inadequate, especially in areas where the microbial bioburden is high (>10^5cfu). Additionally, surface sampling of the patient rooms revealed that bacteria persisted frequently on the nurse call button, patient chair seat, sofabed seat, bin lid, inside door handles, and on floor and bathroom floors despite HPV disinfection (Table II). The persistence of bacteria on high-frequency-touch surfaces may pose a risk of recontamination to nearby surfaces.

<insert Table III near here>

A randomized ward study showed that HPV, a chlorine-releasing agent and peracetic wipes were more effective in removing *C. difficile* spores than ozone, microfibre or steam cleaning.[23] There was no significant difference between the first three methods despite the differences in cost. Comparisons between HPV and aerosolized hydrogen peroxide have suggested that HPV is more effective.[18] A comparison of HPV and aerosolized hydrogen peroxide found uneven distribution for the aerosolized method resulting, in places, in 2 $\log_{10}$ lower eradication of test samples of *C. difficile*, MRSA and *Acinetobacter baumannii*.[18] In the current study, both systems generated HPV, albeit using different starting concentrations of hydrogen peroxide and different modes of generating vapour. Although the starting concentration of hydrogen peroxide used in HPS1 was higher (30%) than in HPS2 (4.9%) (Table III), there was no significant difference in the efficacy of the two HPV systems against in-house BIs. However, the aerial concentrations of HPV achieved by each system were not measured in this evaluation, although this is the subject of future assessments.

Hydrogen peroxide systems need to demonstrate: (i) minimal operational disruption to the healthcare service; (ii) minimal storage requirements when not in use; and (iii) integration

within environmental cleaning requirements in multi-site organizations. HPS1 comprised two units (~65 kg each) of approximately 0.6 x 0.7 x 1.3 m and required an oscillator fan to circulate the hydrogen peroxide generated. HPS2 comprised a single unit (~70 kg) and required less storage space than HPS1 (approximately 0.6 x 0.6 x 1.1 m). Another aspect considered was the need for risk assessments when handling and storing hydrogen peroxide solutions. However, during this study, both parties provided storage of equipment and hydrogen peroxide stock solutions off-site.

The distribution of in-house BI coupons at various sites showed potential limitations in the decontamination efficacy of each system. MRSA (~2–816 cfu) persisted on 40/150 (26.6%) and 37/146 (25.3%) test coupons after treatment with HPS1 and HPS2, respectively (Table III). Despite median recovery of 0 cfu *C. difficile* spores, between 6 and 372 cfu persisted on 8/300 (2.7%) and 21/294 (7.1%) in-house BI coupons exposed to HPS1 and HSP2 treatment, respectively. In rooms treated with HPS1, *C. difficile* persisted on 7/8 sampling occasions (87.5%) behind the door to the isolation room (floor level). When rooms were disinfected using HPS2, *C. difficile* persisted most frequently underneath the bed (Figure 1, BI Position 2) and window frame in 6/21 cases (28.6%). Previous studies evaluating hydrogen peroxide aerial decontamination systems have demonstrated kill curves producing an initial linear phase in efficacy followed by a pronounced trailing phase, suggesting that low numbers of organisms are likely to remain on surfaces unless the cycle duration is increased.[24] However rapid turnaround is essential for clinical ward management, and the risks posed by the numbers of organisms persisting is low.

A consideration when choosing a disinfectant is the inadvertent de-activation of the active agent (i.e. hydrogen peroxide) by the target organism (e.g. catalase production).[24] Both MRSA and *K. pneumoniae* used in the study were catalase producers. Although MRSA

persisted on some coupons, *K. pneumoniae* was eradicated from all coupons using either system (Table III).

Hydrogen peroxide decontamination systems have been criticized because of the need for patients and staff to vacate the room during the procedure, the need for well-trained and informed staff to operate them, the high cost and the long turnaround time (3–4 h).[25] Therefore, some bed areas are not amenable to their use. Furthermore, the environment may be recontaminated rapidly by a colonized patient. However, both systems effectively reduced bacterial contamination to very low levels, even when surfaces were heavily soiled (10% BSA or synthetic body fluid). The presence of bacteria on the outside door handles of the isolation rooms in the majority of cases (90%) following HPV disinfection highlights that correct hand-hygiene and infection-control procedures are essential in preventing the transmission of pathogenic organisms into a decontaminated room. Long-term studies are awaited to confirm whether or not regular use of HPV reduces the prevalence of hospital-acquired infections.

Conflict of interest statement

None declared.

Funding sources

This research was funded by unrestricted grants from Bioquell and Hygiene Solutions, and supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. Equipment was provided free of charge by Bioquell, Andover, UK (Bioquell Q10) and Hygiene Solutions, Kings Lynn, UK (Deprox). Financial support was provided equally by both parties to subsidize microbiological media costs. However, neither party was involved in the study design, research outcome or writing and editing of the

manuscript. APRW was supported, in part, by the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

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Table I

Location of 22 sites sampled in single isolation rooms before and after hydrogen peroxide vapour (HPV) decontamination. Sites represent high-frequency-touch surfaces and areas that are difficult to access or difficult to clean (manually) in patient rooms

Sampling location	Sampling code	Sample point description[a]
Side-room/bay area		
	1	Bed frame (right-hand side)
	2	Footboard
	3	Bed control panel
	4	Nurse call button
	5	Patient chair arm (right-hand side)
	6	Patient chair seat
	7	Sofabed seat
	8	Bin lid
	9	Patient entertainment system
	10	Suction container
	11	Suction tubing
	12	Inside door handle
	13	Outside door handle
	14	Corner of main room (floor) behind entrance door
Patient bathroom		
	15	Inside door handle
	16	Outside door handle
	17	Toilet assist bar (wall-mounted)
	18	Toilet flush
	19	Sink tap handle

20	Shower head handle
21	Shower curtain
22	Bathroom floor corner (opposite shower tray)

[a]Spot sampling (25 cm^2) before and after HPV decontamination was performed in adjacent areas per sampling point to avoid resampling of the same surface.

Table II

Median [interquartile range (IQR)] numbers of total aerobic bacteria recovered from surfaces from 22 sites in side-rooms before and after whole-room decontamination with one of two hydrogen peroxide vapour systems

Median (IQR) numbers of colony-forming units recovered from surfaces in single rooms (*N*=10) treated with hydrogen peroxide vapour										
	Hydrogen peroxide system 1					Hydrogen peroxide system 2				
Site	Pre-exposure		Post-exposure		Persistence % (P/tot)[a]	Pre-exposure		Post-exposure		Persistence %(P/tot)[a]
1 Bed frame (right-hand side)	5.5	(3.3–18.0)	0.0	(0.0–1.0)	40 (4/10)	14.0	(1.8–41.3)	0.0	(0.0–0.0)	22 (2/9)
2 Footboard	7.0	(3.0–22.5)	0.5	(0.0–1.0)	50 (5/10)	14.5	(9.5–34.0)	0.0	(0.0–3.3)	44 (4/9)
3 Bed control panel	24.0	(11.3–24.5)	0.0	(0.0–0.0)	20 (2/10)	33.5	(1.8–56.5)	0.5	(0.0–2.0)	56 (5/9)
4 Nurse call button	21.5	(8.5–34.8)	0.5	(0.0–4.0)	50 (5/10)	37.0	(29.0–123.3)	3.5	(1.0–9.8)	80 (8/10)
5 Patient chair arm (right-hand side)	18.0	(11.5–24.5)	0.0	(0.0–0.8)	30 (3/10)	20.0	(13.0–23.0)	1.0	(1.0–3.0)	78 (7/9)
6 Patient chair seat	64.5	(33.8–68.8)	1.5	(1.0–3.0)	90 (9/10)	82.0	(36.0–182.0)	7.0	(6.0–47.0)	100 (9/9)
7 Sofabed seat	17.0	(6.0–56.0)	1.0	(0.0–2.0)	56 (5/9)	49.0	(6.0–89.0)	6.0	(1.0–13.0)	89 (8/9)
8 Bin lid	30.0	(23.5–44.5)	1.0	(0.0–3.5)	60 (6/10)	52.0	(38.3–62.0)	1.5	(1.0–2.0)	30 (3/10)

9 Patient entertainment system	5.0	(2.0–20.0)	0.0	(0.0–1.0)	44 (4/9)	4.5	(1.5–13.3)	0.0	(0.0–0.8)	30 (3/10)
10 Suction container	35.0	(16.3–45.8)	0.0	(0.0–0.0)	20 (2/10)	14.0	(6.3–53.0)	0.0	(0.0–0.0)	20 (2/10)
11 Suction tubing	11.0	(10.0–14.0)	0.0	(0.0–0.0)	11 (1/9)	8.5	(4.3–22.0)	0.0	(0.0–1.8)	40 (4/10)
12 Inside door handle	13.0	(6.0–26.3)	1.5	(0.3–2.8)	70 (7/10)	22.5	(10.5–47.8)	1.0	(0.0–2.8)	60 (6/10)
13 Outside door handle	14.5	(7.25–25.3)	9.5	(5.8–20.8)	90 (9/10)	26.0	(16.3–56.8)	5.0	(1.5–17.8)	90 (9/10)
14 Corner of main room (floor) behind entrance door	352.5	(193.3–500.0)	3.0	(2.3–5.3)	100 (10/10)	238.0	(135.3–500.0)	3.5	(1.0–5.0)	90 (9/10)
15 Inside door handle (toilet)	38.0	(12.3–74.8)	1.0	(0.0–1.8)	60 (6/10)	51.0	(14.3–61.3)	0.0	(0.0–0.0)	20 (2/10)
16 Outside door handle (toilet)	8.0	(7.0–22.3)	0.0	(0.0–0.8)	30 (3/10)	28.0	(13.8–62.5)	0.0	(0.0–0.8)	30 (3/10)
17 Toilet assist bar	18.0	(13.8–26.8)	0.0	(0.0–1.0)	40 (4/10)	13.0	(1.3–23.5)	1.0	(0.0–2.8)	60 (6/10)
18 Toilet flush	39.0	(31.5–53.8)	0.5	(0.0–1.0)	50 (5/10)	11.5	(7.3–15.3)	0.0	(0.0–0.0)	20 (2/10)
19 Sink tap handle	25.5	(7.8–42.8)	0.0	(0.0–1.0)	40 (4/10)	40.0	(9.8–49.3)	0.0	(0.0–0.0)	10 (1/10)
20 Shower head handle	10.5	(4.3–31.3)	0.0	(0.0–0.8)	30 (3/10)	25.5	(16.0–62.8)	0.0	(0.0–1.5)	30 (3/10)
21 Shower curtain	10.0	(3.8–15.5)	0.0	(0.0–4.0)	40 (4/10)	5.0	(2.3–12.3)	0.5	(0.0–1.0)	50 (5/10)
22 Bathroom floor corner (opposite shower tray)	500.0	(223.0–500.0)	3.5	(0.8–5.5)	70 (7/10)	223.5	(125.0–271.5)	1.0	(0.3–0.3)	70 (7/10)

P, number of sites where bacteria detected; Tot, total number of sites sampled.

[a]Percentage of sites where >0 cfu bacteria recovered after disinfection calculated as:

%= [number of positive sites/total number of sites sampled]*100.

Table III

Efficacy of two hydrogen peroxide systems to decontaminate single isolation rooms. Efficacy was determined from differences between median [interquartile range (IQR)] numbers of bacteria or spores recovered from in-house biological indicator (BI) coupons. All test organisms were evaluated with low soiling, heavy soiling [meticillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae*] or synthetic faeces (*Clostridium difficile* spores). BI coupons were placed at five different locations per room

	Hydrogen peroxide system 1		Hydrogen peroxide system 2	
	cfu remaining (IQR)[a]	Reduction log10 cfu (IQR)	cfu remaining (IQR)[a]	Reduction log10 cfu (IQR)
MRSA				
BI location[b]				
Low soiling (0.03% BSA)				
1	<2	6.36 (6.28–6.52)	<2	6.29 (5.98–6.50)
2	<2 (0–2)	6.42 (6.30–6.48)	<2	6.29 (5.91–6.52)
3	<2	6.41 (6.32–6.48)	<2	6.36 (5.91–6.44)
4	<2 (0–2)	6.34 (6.28–6.38)	<2	6.31 (5.73–7.00)
5	<2 (0–4)	6.34 (6.26–6.47)	<2	6.24 (6.00–6.36)
Heavy soiling (10% BSA)				
1	<2	6.29 (6.14–6.55)	18 (0–172)	6.49 (6.35–6.66)
2	<2 (0–2)	6.26 (6.12–6.53)	0 (0–296)	6.44 (6.20–6.67)
3	<2	6.25 (6.07–6.42)	4 (0–488)	6.34 (6.31–6.48)
4	<2 (0–4)	6.30 (6.10–6.38)	<2	6.35 (6.11–6.55
5	<2 (0–4)	6.27 (6.10–6.45)	0 (0–816)	6.37 (6.28–6.81)
K. pneumoniae				
BI location[b]				
Low soiling (0.03% BSA)				
1	<2	6.40 (6.20–6.66)	<2	6.34 (5.73–6.53)
2	<2	6.35 (6.23–6.66)	<2	6.37 (5.81–6.53)
3	<2	6.31 (6.21–6.58)	<2	6.26 (5.85–6.62)

4	<2	6.37 (6.20–6.52)	<2	6.23 (5.76–6.67)
5	<2	6.38 (6.28–6.61)	<2	6.41 (5.80–6.70)
	Heavy soiling (10% BSA)			
1	<2	6.32 (6.26–6.65)	<2	6.39 (5.86–6.62)
2	<2	6.40 (6.26–6.65)	<2	6.30 (5.85–6.42)
3	<2	6.31 (6.23–6.65)	<2	6.38 (5.85–6.55)
4	<2	6.39 (6.24–6.61)	<2	6.28 (5.85–6.63)
5	<2	6.30 (6.25–6.70)	<2	6.43 (5.91–6.65)
***C. difficile* 027 spores**				
BI location[b]				
	Low soiling (0.03% BSA)			
1	<2	5.24 (5.00–5.44)	<2	5.26 (5.05–5.48)
2	<2	5.18 (5.01–5.46)	<2	5.34 (5.06–5.49)
3	<2	5.30 (5.06–5.48)	<2	5.31 (5.07–5.49)
4	<2	5.27 (4.92–5.60)	<2	5.39 (4.96–5.51)
5	<2	5.30 (5.00–5.45)	<2	5.23 (5.09–5.47)
	Heavy soiling (body fluid)			
1	<2	5.31 (5.12–5.47)	<2	5.19 (5.02–5.42)
2	<2	5.25 (5.10–5.60)	<2	5.39 (5.08–5.49)
3	<2	5.31 (5.12–5.52)	<2	5.14 (5.02–5.44)
4	<2	5.34 (5.05–5.51)	<2	5.24 (5.00–5.39)
5	<2	5.29 (5.08–5.58)	<2	5.22 (5.09–5.38)

BSA, bovine serum albumin; cfu, colony-forming units.

[a]Counts below the theoretical detection limit (2 cfu) are denoted as <2.

[b]BI coupons were placed in one of five locations as described in Figure 1.

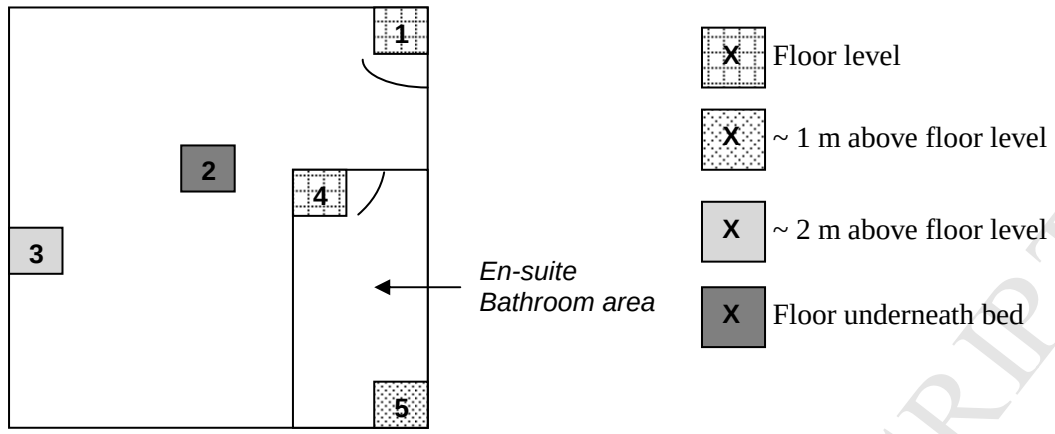

Figure 1. Location of sample points to position microplates containing control and test (exposed) coupons during each hydrogen peroxide vapour (HPV) decontamination cycle. Sample points were arranged to assess the effectiveness of the HPV decontamination system against contamination (biological indicators) in difficult-to-access areas and at various heights within the room. Sample point description: 1, corner of room (floor level) behind main door; 2, underneath bed (floor level); 3, window frame approximately 2 m above floor; 4, corner of bathroom (floor level) behind door; 5, shelf in shower unit approximately 1 m above floor.