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Environmental contamination and airborne microbial counts: a role for hydroxyl radical disinfection units?

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SUMMARY

Environmental contamination is thought to play a role in the spread of infection in hospitals and there has been increased interest in novel air disinfection systems in preventing infection. In this study the efficacy of a hydroxyl radical air disinfection system (Inov8 unit) in reducing the number of airborne bacteria was assessed in a clinical setting. Environmental contamination was assessed using settle plates and air samples in three settings: (1) non-clinical room; (2) non-clinical room with defined activity; and (3) single intensive care unit cubicle. A comparison of air counts and environmental contamination rates was made with the Inov8 units on and off. The Inov8 unit produced an overall reduction in both air sample and settle plate counts in each setting ($P < 0.001$, Wilcoxon signed-rank test). There was a mean reduction in air sample counts of 26%, 39% and 55% for settings 1, 2 and 3 respectively. The corresponding reductions in settle plate counts were 35%, 62% and 54%. These results suggest that this type of novel air disinfection may have a role in improving air quality and reducing environmental contamination within clinical isolation rooms. Further work is required to assess the effect on specific pathogens, and to establish whether this will reduce the risks of patients and/or healthcare workers acquiring such pathogens from the environment.

Introduction

There is increasing interest in new technologies for reducing microbial environmental contamination within healthcare settings. Several studies have suggested that environmental contamination may play a role in the spread of infection.[1,2] A number of air decontamination products have been shown to reduce environmental contamination in patient isolation rooms, including a portable high efficiency particulate air (HEPA) filtration unit and a dry mist hydrogen peroxide delivery system.[3,4]

The Inov8 air disinfection (AD) unit is a novel air purifying system against microbial aerosols that could potentially be used in healthcare settings. The device produces hydroxyl radicals from a reaction of ozone and water vapour catalysed by an olefin (D-limonene).[5] Hydroxyl radicals are normally present in open air with the main source of hydroxyl in air being the photolysis of ozone – this is known as the 'open air factor'.[6] Hydroxyl radicals have also been shown to possess disinfection characteristics.[6,7]

Hydroxyl radicals have been implicated in the oxidation of a large number of biomolecules, including protein and DNA.[8] The mechanism of DNA damage is through DNA strand breakage.[9] Once formed, the hydroxyl radical is likely to travel only a short distance before it encounters an oxidisable substrate. This reaction, in turn, can result in a free radical cascade and cause cell injury at sites distant from where the initial free radical reaction occurred.[8] In addition, neutrophils and other phagocytes are known to manufacture hydroxyl radicals as part of the human immune system to kill invasive micro-organisms, with such radicals being found in inflamed tissue.[9]

In one study the Inov8 device was tested in an environmental chamber against aerosols of an MS-2 coliphage (an unenveloped single-stranded RNA coliphage) and *Staphylococcus epidermidis*. The device was shown to significantly reduce the concentration of both airborne microbes by a factor of 5 $\log_{10}$ within 1 h.[10]

The aim of this study was to evaluate the effectiveness of the Inov8 AD unit in reducing total airborne microbial counts and environmental contamination in a clinical setting.

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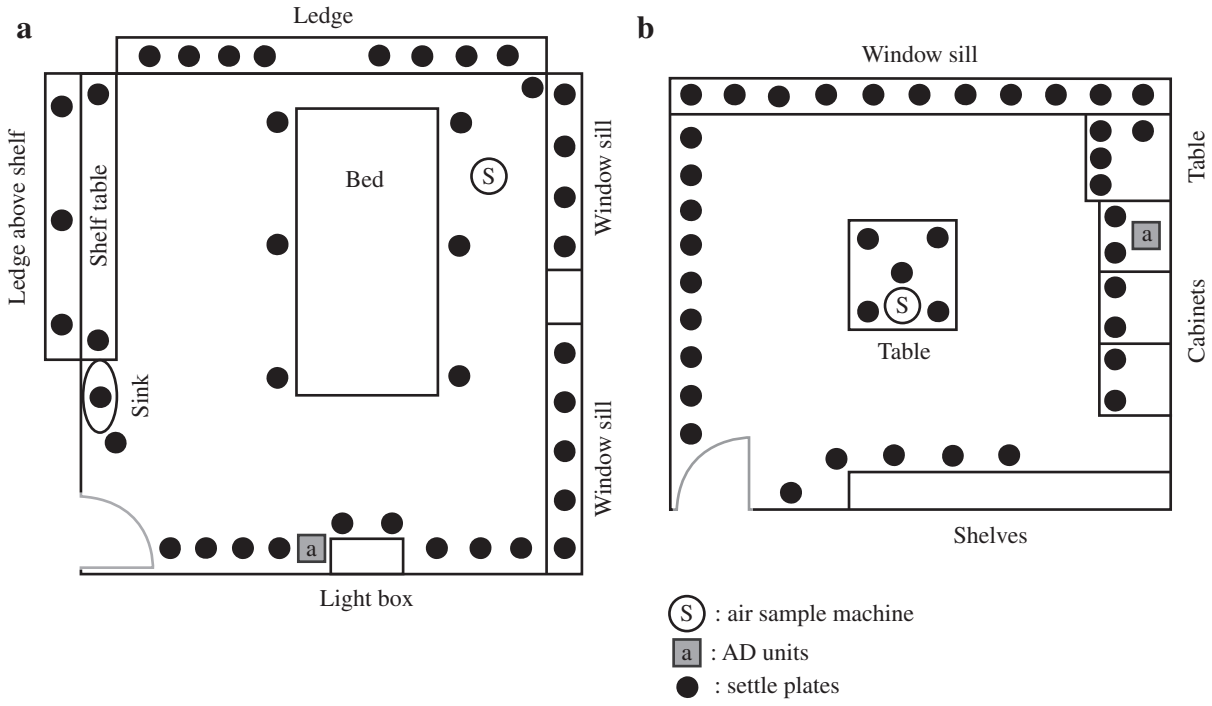

Figure 1. Floor plan of the single intensive care unit (ICU) cubicle (a) and the non-clinical room (b). AD, air disinfection unit.

Methods

Inov8 air disinfection unit

Inov8 air disinfection units were kindly provided by Inov8 Science Ltd (Buckingham, UK). Each machine holds a sealed cartridge of ozone and water vapour that is catalysed by olefin. One cartridge lasts about 14 weeks: cartridge changes were made during the study.

Exposure of agar to hydroxyl radicals

To establish whether hydroxyl radicals might affect the growth medium, tryptone soya agar (TSA), a comparison of bacterial growth was made between six TSA plates that had been exposed to hydroxyl radicals and six control TSA plates. The open TSA plates (both test and control) were placed in a Class I laminar flow cabinet, 1 m from an AD unit (either on or off), within a clean preparation facility. The laminar flow cabinet had an airflow velocity of 45 m/s delivering HEPA-filtered air. Thereafter each plate was inoculated with five drops of 20 μL of Oxoid *Staphylococcus aureus* solution at a concentration of 10^{-6} colony-forming units per millitre determined by the Miles and Misra method.[11] The average total viable count (TVC) from 30 drops was calculated for each group.

Study setting

Over a six-month period the effect of the AD unit was assessed in three settings: (1) a non-clinical room; (2) the same non-clinical room but with defined human activity; and (3) a single occupied intensive care unit (ICU) cubicle.

Non-clinical room

This was an unused office in the microbiology department, with closed windows and no ventilation system. One AD unit was placed 155 mm above floor level (Figure 1). For setting 1, the room was unoccupied except for one person entering to change the agar plate in the air sampler every 15 min, over a 4 h period. For setting 2, the additional defined human activity involved one person fanning a worn laboratory coat with both hands, 10 times, in the centre of the room (next to the AD unit), every 15 min, then leaving. The room was routinely cleaned once a week.

Single ICU cubicle

For setting 3, a single patient-occupied ICU cubicle was sampled during normal clinical activity. One AD unit was mounted to the wall in the cubicle (Figure 1). The windows in the single ICU cubicle were kept closed at all times and the door was only opened to allow entry and exit of staff and relatives. The room was ventilated via two supply and four extraction grills, designed to deliver 12 air changes per hour. The number of people and the activity in the single cubicle was recorded and graded into low (1), moderate (2), or high (3), every 15 min over a 4 h time period (Table I).

Study design

For each setting, eight sets of paired data were collected; four sets with the AD unit on for the first day and off for the next, and four sets with the AD unit off for the first day and on for the next. Airborne bacterial counts were measured using volumetric air sampling. The rate of surface contamination was assessed using agar settle plates. The machine was switched on at least 18 h prior to sampling for the study days with the AD unit on.

Sampling method

On each study day, 17 air samples (volume 1 m^3) were drawn onto standard-sized plates containing TSA, every 15 min, over 4 h, using a portable MAS 100 air sampler (Merck Ltd, Germany) with its position unchanged throughout. Forty standard-sized TSA settle

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Table I
Activity grading system in single intensive care unit (ICU) cubicle

Grade of activity	Examples of activities
Low	Patient asleep or resting One nurse sat at notes trolley Door opened for entry/exit of person(s)
Moderate	Staff moving around patient/equipment Nurse preparing drugs Venepuncture of patient Physiotherapy Examination of patient Patient agitated or moving around in bed One relative by bed
High	Washing/turning/transferring patient Changing bedclothes Cleaning[a] Ward round Two or three relatives by bed Procedure being performed on patient, e.g. bronchoalveolar lavage

[a] ICU cleaning protocol: Cleaning was carried out routinely twice a day and before a new patient was admitted. Two cleaners changed the bins, swept and mopped the floor, and cleaned all surfaces and the washbasin. The disinfectant used was 0.1% sodium hypochlorite. The curtains were changed when a new patient was admitted.

plates were exposed to the air for a total of 4 h. Identical settle plate positions were used on each study day (Figure 1).

Microbial culture

All agar plates were pre-incubated for 24 h, at 35–37 °C, in aerobic conditions before sampling to eliminate plate contamination. After sampling, the plates were immediately incubated at 37 °C under aerobic conditions. At 48 h incubation, the TVC of bacteria on the TSA was counted using an electronic hand-held colony counter (Lab Tek Ltd, Yeovil, UK). For three sets of data (non-clinical room with defined activity) the incubation of the TSA plates was extended to 96 h, in order to assess whether the AD unit might have caused attenuated growth.

Data analysis

Statistical analysis was performed using either StatCalc version 7 or StatPlus version 2009 software. Paired *t*-test analysis was performed on the TVCs from TSA agar with and without prior agar exposure to hydroxyl radicals. The graded activity in the single ICU cubicle was analysed using χ^2-test.

The TVCs obtained from the air samples and settle plates were analysed in two ways. First, the mean TVCs were ascertained for each study day, with the AD unit on and off respectively. The mean percentage reduction when the AD unit was on was calculated from the corresponding paired study day with the AD unit off. The median, interquartile range and range of the combined data for each of the three settings were compared using box-and-whisker plots, and the median percentage reductions calculated. In addition, the data from all study days were combined and the overall mean TVCs compared between the days with the AD unit on and off. The mean TVCs obtained from corresponding air samples and settle plates were compared by calculating the Pearson correlation coefficient.

Second, the data were analysed by using paired analyses. Each air sample was paired in time to the corresponding air sample of the paired study day. Likewise each settle plate was paired by position to the corresponding settle plate in the paired study day. A comparison of the TVCs of the entire dataset (136 paired air samples; 320 paired settle plates) was performed using the Wilcoxon signed-rank test. The percentage reduction in TVC with the AD unit on was also calculated for each of these paired samples, and the mean percentage difference [with 95% confidence intervals (CIs)] calculated.

Results

Exposure of agar to hydroxyl radicals

There was no statistically significant difference between the mean TVCs of the TSA agar plates previously exposed to the AD unit compared to controls [mean TVC: 29.9 (SD: 2.98) vs mean TVC: 30.2 (SD: 2.34); $P = 0.73$].

Effect of the Inov8 AD unit on bacterial environmental contamination

The mean TVCs from air samples for each study day and the mean percentage reduction found when the AD unit was on is shown in Table II. The corresponding data for settle plate results is shown in Table III. The median, interquartile range and range of the combined data for both air samples and settle plates for all three settings is shown in Figure 2.

Table II
Comparison of air sample total viable counts (TVCs) obtained with the air disinfection (AD) unit on and off in each setting

Study days	Non-clinical room			Non-clinical room with defined activity			Single ICU cubicle		
	Mean[a] air TVC/m^3		Mean % reduction (95% CI)	Mean[a] air TVC/m^3		Mean % reduction (95% CI)	Mean[a] air TVC/m^3		Mean % reduction (95% CI)
	AD off	AD on		AD off	AD on		AD off	AD on	
1 and 2	179	52	71%	237	68	71%	349	162	53%
3 and 4	41	37	11%	475	180	62%	323	157	51%
5 and 6	51	34	33%	432	428	1%	110	104	6%
7 and 8	41	41	0%	205	152	26%	79	41	48%
9 and 10	655	491	25%	112	135	−17%	42	56	−24%
11 and 12	410	359	13%	114	175	−34%	395	56	86%
13 and 14	165	177	−7%	96	140	−31%	90	38	58%
15 and 16	105	33	68%	525	72	86%	73	46	36%
All study day samples combined (*P*-value)[b]	206	153	26% ($P < 0.001$)	275	169	39% ($P < 0.001$)	183	83	55% ($P < 0.001$)
Paired analysis[c]	–	–	21% (14–28%)	–	–	21% (13–29%)	–	–	19% (11–27%)

ICU, intensive care unit.
[a] Mean TVC from 17 × 1 m^3 air samples taken on each study day (mean TVC from all 136 air samples for the combined data).
[b] Wilcoxon signed-rank test.
[c] Mean percentage difference in TVCs from 136 consecutive paired air samples taken over the 16 study days.

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Table III
Comparison of settle plate total viable counts (TVCs) obtained with the air disinfection (AD) unit on and off in each setting

Study days	Non-clinical room			Non-clinical room with defined activity			Single ICU cubicle		
	Mean[a] settle plate TVC/4 h exposure		Mean % reduction (95% CI)	Mean[a] settle plate TVC/4 h exposure		Mean % reduction (95% CI)	Mean[a] settle plate TVC/4 h exposure		Mean % reduction (95% CI)
	AD off	AD on		AD off	AD on		AD off	AD on	
1 and 2	20	11	44%	22	13	42%	93	32	65%
3 and 4	13	8	42%	216	27	87%	94	31	67%
5 and 6	9	5	46%	139	69	48%	52	40	23%
7 and 8	11	8	24%	64	65	−1%	35	33	5%
9 and 10	94	68	28%	33	30	9%	14	18	−23%
11 and 12	73	38	48%	93	106	−13%	118	32	73%
13 and 14	44	41	8%	32	69	−54%	29	18	37%
15 and 16	24	8	66%	469	31	93%	71	28	60%
All study day samples combined (*P*-value)[b]	36	23	35% ($P < 0.001$)	134	51	62% ($P < 0.001$)	63	29	54% ($P < 0.001$)
Paired analysis[c]	–	–	35% (32–39%)	–	–	27% (21–32%)	–	–	38% (33–42%)

ICU, intensive care unit.
[a] Mean TVC per 4 h exposure from 40 settle plates per study day (mean TVC from all 320 settle plates for the combined data).
[b] Wilcoxon signed-rank test.
[c] Mean percentage difference in TVCs from 320 paired settle plate readings.

In each setting the AD unit produced an overall reduction in airborne and settle plate counts when the data were combined. For air samples, the mean reduction in TVC for settings 1, 2 and 3 was 26%, 39% and 55% respectively (Table II). The corresponding mean reductions in settle plate counts were 35%, 62% and 54% (Table III). However, the mean percentage reduction in TVC varied across each of the 24 paired study days. For air samples, the mean percentage reduction with the AD unit on varied from −34% to 86%; a positive reduction was found on 17/24 paired days, there was no difference on 2/24 paired days, and there was actually an increase (negative percentage reduction) in mean TVCs on 5/24 paired days. For settle plates the mean percentage reduction ranged from −23% to 93%, with a positive reduction in 20/24 paired study days.

There was a correlation between the air sample and settle plate results. In each setting, the study day (AD off) with the highest mean TVC from air samples also had the highest mean TVC from settle plates. Similarly study days with low mean air TVCs generally had lower settle plate counts (Tables II and III). Overall the mean percentage reductions in TVC for air samples and settle plates for each paired study day were highly correlated ($r = 0.79$; Pearson correlation coefficient).

Figure 2 shows the reduction in both median and maximum TVC values for the combined data when the AD was on in each setting. The percentage reduction in median TVC from air samples was 57%, 42% and 39% for settings 1, 2 and 3 respectively. The corresponding percentage reduction in median TVC from settle plates was 33%, 54% and 54%.

The overall percentage reduction found in mean and median TVCs in each setting when the AD unit was on was supported by the non-parametric paired analyses (Wilcoxon signed-rank test) of the 136 and 320 paired air samples and settle plates. In each setting, there was a statistically significant reduction in TVCs from air sampling and settle plates ($P < 0.001$ in all cases; Tables II and III). The mean of the percentage reductions calculated by analysis of all the individual paired sets of data was somewhat less (19–21% for air samples; 27–38% for settle plates) than the corresponding overall reduction calculated by the non-paired analyses (Tables II and III). However, none of the 95% CIs included zero.

The activity in the single ICU room was graded from low (1) to high (3). The number of episodes for each grade of activity was recorded with the AD unit on and off. Episodes of activity according to grade and machine status were dependent according to χ^2-testing ($P = 0.013$). There were equal amounts of high grade activity between the two groups, but there were more episodes of medium grade activity with the AD unit off and more episodes of low grade activity with the AD unit on (Table IV).

Extended incubation

Prolonging the TSA plate incubation to 96 h resulted in a small increase in the TVCs. The air sample mean TVCs were 246.5/m^3 (48 h) and 247.6/m^3 (96 h) with the AD off, and 137.7/m^3 (48 h) and 139.9/m^3 (96 h) with the AD on. For settle plates, the mean TVCs were 146.3/4 h (48 h) and 147.32/4 h (96 h) with the AD off, and 51.9/4 h (48 h) and 53.6/4 h (96 h) with the AD on.

Discussion

It was important to ensure that the reduction in TVCs measured from our samples with the AD unit on were from the effects of the hydroxyl radicals on the viability of the airborne micro-organisms, rather than damage or alteration to the growth medium, thus potentially reducing the ability of the TSA to recover them. However, we found that prior exposure of TSA to hydroxyl radicals produced no statistically significant effect on bacterial recovery, although it could be argued that the higher air velocity within the laminar flow cabinet might have reduced the concentration of hydroxyl radicals, resulting in these test plates having less exposure to the hydroxyl radicals, compared to the plates used in the main study.

The air sampling results showed a wide variation in bacterial counts in all three settings. The air samples were each taken over a relatively short time period and it is known that the microbial quality of air can vary due to the occurrence of individual events that alter the quality of the air.[12] Nevertheless the results did show an overall mean reduction in airborne bacterial counts of between 26% and 55% with the AD unit on. As the data were not normally distributed, we also compared the median and ranges of the data sets. This showed a similar overall reduction in median counts (39–57%) and a reduction in the maximum counts. These reductions were highly significant ($P < 0.001$) using non-parametric paired analyses.

There was somewhat less variation with the settle plate data compared to air samples. Again there was an overall reduction in mean and median settle plate counts of 35–62% and 33–54% respectively ($P < 0.001$). Settle plates represent the rate of bacterial surface contamination and are thought to be a more reliable measurement of environmental burden than air samples because individual events are lost.[12] However, we found that there was

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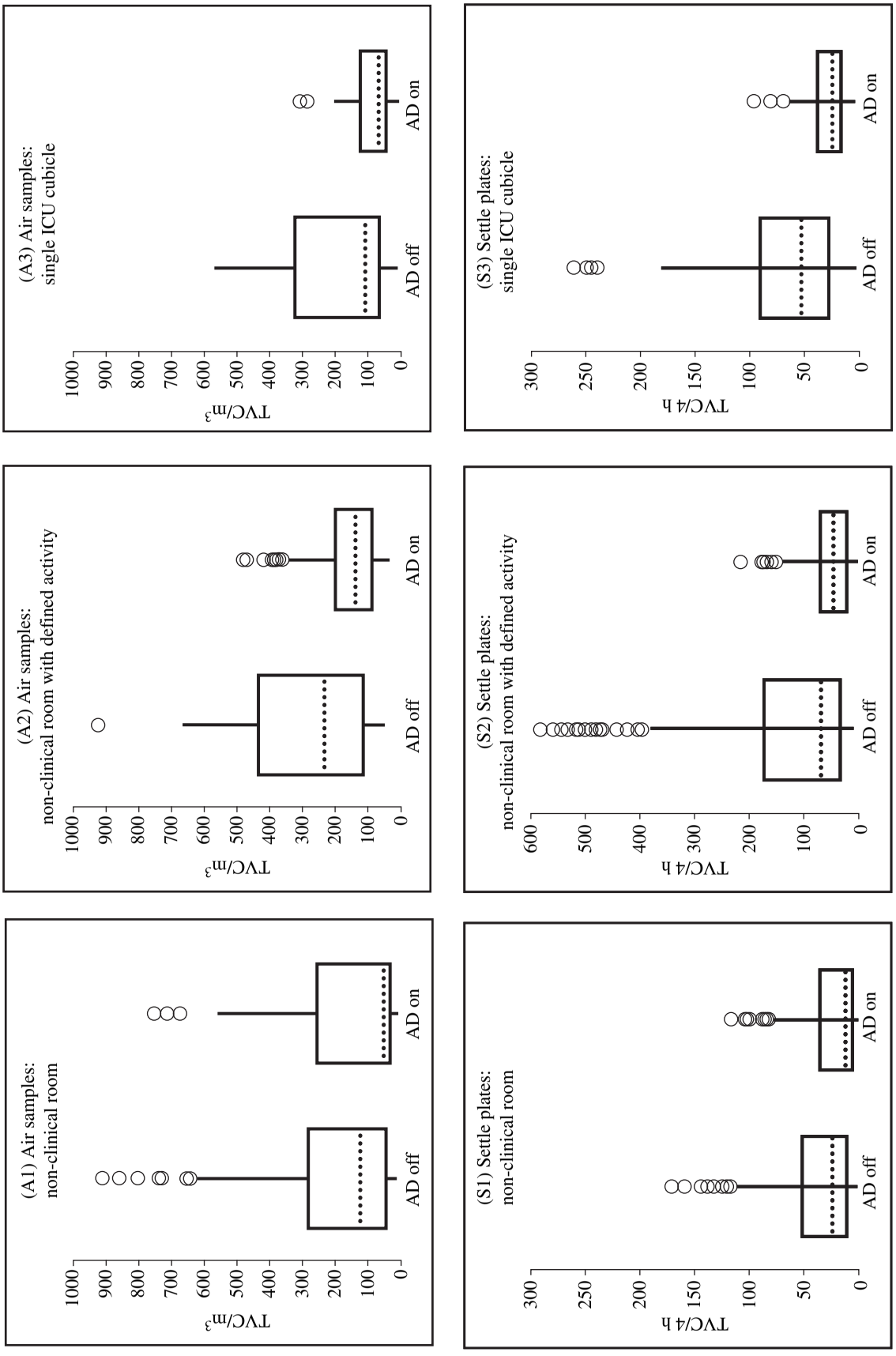

Figure 2. Box-and-whisker plots showing total viable count (TVC) of bacteria in the air (air samples) for three settings: (A1) non-clinical room, (A2) non-clinical room with defined activity and (A3) single intensive care unit (ICU) cubicle; and the TVC of bacteria for settle plates for three settings: (S1) non-clinical room, (S2) non-clinical room with defined activity and (S3) single ICU cubicle. The box represents the interquartile range (IQR) (25th–75th percentiles), the horizontal line the median value, the whiskers the range, outliers are represented by '○' (observations 1.5 × IQR outside central box). AD, air disinfection unit.

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Table IV
Number of episodes of activity according to grade recorded every 15 min over 4 h for all 16 study days in the single intensive care unit cubicle

Grade	Machine status	
	AD off	AD on
Low	27	47
Medium	55	38
High	37	34
Total	119	119

AD, air disinfection unit.

quite a strong correlation between the overall air sample results and the settle plate data ($r = 0.79$).

We also calculated the mean and 95% CIs of all the percentage differences of all the individual paired data. These overall reductions were lower (27–38%) than the overall reductions calculated by the non-paired analysis, but the 95% CIs (range: 21–42%) also suggested a statistically significant effect. The lower calculated reduction by this method was partly due to the percentage change in each of the paired readings having an equal effect on the overall mean, including paired readings where the absolute difference was very small, yet the percentage difference quite high. The AD unit appeared to have greater effect at reducing counts when the air and settle plate counts were high, and there was less demonstrable effect when the air counts were low.

Although the overall results showed a reduction in air and settle plate counts with the AD unit on, there were some paired study days when the counts appeared to increase. The paired study days were only 24 h apart, but the background environmental conditions may not have been the same, and we could not control for factors such as temperature and humidity. This may have caused chance variation in background counts. There was quite a lot of variation in the background counts within the non-clinical room with AD unit off, which was difficult to explain. However, of the eight paired study days in the clinical setting (ICU cubicle), there was a significant reduction in air and settle plate counts with the AD unit on, for all days where the paired study days (AD unit off) had relatively high counts. The one paired set of data that showed an increase in counts (both air and settle plates) with the AD unit on had relatively low counts on both days. One confounding factor in this setting might have been the degree of human activity. Whereas the amount of high grade activity was equal between study days, there was significantly more medium grade activity on the study days with the AD unit off, and this could partly explain the lower counts found with the AD unit on.

We incubated the TSA agar for 48 h and then enumerated the aerobic TVC. There was no significant increase in TVCs after 96 h incubation. This suggests that the reduction in counts due to the AD unit was probably more due to a bactericidal rather than bacteriostatic effect, but further *in vitro* work would be required to establish whether the primary mode of action of the AD unit is bactericidal.

Our results showed a significant overall reduction in airborne contamination in the patient-occupied clinical isolation room of around 50% as assessed by both repeated air-sampling and settle plates. This reduction is several orders of magnitude less than the 5 $\log_{10}$ reduction achieved against an aerosol of *S. epidermidis* in an environmental test chamber.[10] This could be due to several factors including the different physical nature of airborne pathogens. Many of the airborne bacteria in a clinical setting are found as microcolonies on skin squames, and hydroxyl radicals may be less effective at killing these compared to an artificially generated aerosol. There has been one other smaller study (published only in abstract form) on the evaluation of the Inov8 AD unit in a clinical setting. This showed a significant reduction in settle plate bacterial counts in both an unoccupied (79% reduction) and occupied (72% reduction) burns ICU isolation room.[13]

The advantage of the Inov8 system is that it can be used to improve microbial air quality in patient-occupied rooms. Although we have shown a statistically significant reduction in total aerobic counts with this system, the magnitude of the effect was less than expected and further work is required to establish whether a reduction of around 50% will translate to a significant reduction in overall environmental contamination and reduced risks of cross-infection. This reduction was less than achieved, for example, with a portable HEPA filtration unit, which produced a 75–90% reduction in meticillin-resistant *Staphylococcus aureus* (MRSA) contamination rates (measured by settle plates).[3] One advantage of the Inov8 unit is that it is completely silent and much smaller than other systems. Further work is now required to examine the effects of the Inov8 system in reducing environmental contamination with specific pathogens such as MRSA, *Clostridium difficile*, aspergillus and norovirus.

Despite the potential benefits of decontaminating clinical areas, the long term effects of hydroxyl radicals on human health remain to be established and this also requires further investigation.

In conclusion, this study suggests that the Inov8 hydroxyl radical AD unit may have a role in reducing the environmental burden of bacteria in a high risk clinical isolation room, but more data are required to demonstrate whether the technology will be of benefit alongside our current infection prevention procedures.

Acknowledgements

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Conflict of interest statement

Since the research was completed, Dr T. Boswell has become a member of The Inov8 Scientific Advisory Board.

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