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Temperature-controlled airflow ventilation in operating rooms compared with laminar airflow and turbulent mixed airflow

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SUMMARY

Aim: To evaluate three types of ventilation systems for operating rooms with respect to air cleanliness [in colony-forming units (cfu/m^3)], energy consumption and comfort of working environment (noise and draught) as reported by surgical team members.

Methods: Two commonly used ventilation systems, vertical laminar airflow (LAF) and turbulent mixed airflow (TMA), were compared with a newly developed ventilation technique, temperature-controlled airflow (T_cAF). The cfu concentrations were measured at three locations in an operating room during 45 orthopaedic procedures: close to the wound (<40 cm), at the instrument table and peripherally in the room. The operating team evaluated the comfort of the working environment by answering a questionnaire.

Findings: LAF and T_cAF, but not TMA, resulted in less than 10 cfu/m^3 at all measurement locations in the room during surgery. Median values of cfu/m^3 close to the wound (250 samples) were 0 for LAF, 1 for T_cAF and 10 for TMA. Peripherally in the room, the cfu concentrations were lowest for T_cAF. The cfu concentrations did not scale proportionally with airflow rates. Compared with LAF, the power consumption of T_cAF was 28% lower and there was significantly less disturbance from noise and draught.

Conclusion: T_cAF and LAF remove bacteria more efficiently from the air than TMA, especially close to the wound and at the instrument table. Like LAF, the new T_cAF ventilation system maintained very low levels of cfu in the air, but T_cAF used substantially

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less energy and provided a more comfortable working environment than LAF. This enables energy savings with preserved air quality.

Introduction

Air with a low concentration of viable bacteria in the operating room (OR) has long been known as one of the key factors to prevent deep surgical site infections (SSI) [1,2]. With the increasing occurrence of antibiotic-resistant bacteria that cause SSI, reliance on antibiotic prophylaxis cannot continue. Thus, other measures, such as ventilation of the OR, have to be as efficient as possible. Charnley and Eftekhar [2] improved the microbiological air quality by introducing low-turbulence displacement airflow facilities, resulting in a reduction of the incidence of infection from 8.9% to 1.3% in orthopaedic surgeries. The ventilation air introduced into the room is filtered and free from bacteria. This means that the main sources of airborne bacteria in the OR are particle shedding from the surgical team and from outside air that enters during door openings.

Traditionally, two main types of ventilation have been used to provide low levels of colony-forming units (cfu) in the OR air: laminar airflow (LAF) and turbulent mixed airflow (TMA). In most studies that measure airborne bacterial loads, LAF ventilation appears to be superior to TMA ventilation [3—6]. However, in recent years, epidemiological registry studies have shown that the risk of SSI after surgery in LAF is equal to TMA, or even higher [7,8]. For this reason, the World Health Organization (WHO) stated that LAF should not be used for total arthroplasty surgery [9]. The WHO recommendation is conditional as there is very limited evidence on the efficiency of different ventilation systems with regard to the incidence of SSI [9]. Consequently, there is an urgent need for more evidence to enhance and facilitate decision making about ventilation techniques when building new hospitals and renovating old ones.

The aim of this study was to compare three ventilation techniques for ORs, focusing on evaluation of a new technique that uses temperature-controlled airflow (T_cAF). T_cAF was compared with conventional LAF and TMA ventilation systems regarding the amount of airborne cfu in the OR, energy consumption and comfort of working environment. Other known parameters reported to affect the cfu count were also included: number of staff in the OR, number of door openings, and duration of surgery [5,10—14].

Materials and methods

Study design

Measurements were taken in three ORs between January 2015 and February 2016 at the Orthopaedic Surgery Department, Helsingborg General Hospital, Helsingborg, Sweden. This is an acute care hospital where approximately 2500 orthopaedic surgical procedures are performed annually. The only difference between the ORs was the type of ventilation system: TMA, LAF or T_cAF. In total, 45 operations were included (15 performed in each OR). The procedures were the same in each OR: seven wrist fractures, two shoulder arthroscopies and six hip fracture fixations. During all operations, the staff wore similar clothing of mixed material (69% cotton, 30% polyester, 1% carbon fibre; Mertex P-3477, Mercan AB, Skanör, Sweden) with wristlets at ankles, upper arms and neckline, the shirt tucked in the trousers, and a disposable surgical hood tucked in the neck.

Ventilation systems

The different airflows of the three OR ventilation systems (TMA, LAF and T_cAF) (Figure 1) were modelled using computational fluid dynamic (CFD) models, shown in Figure 2. CFD is a branch of fluid mechanics that uses applied mathematics, physics and computational power to study fluid flows. LAF operated at the highest airflow rate (12,000 m^3/h), which also created higher airflow speeds (Figure 2c—d). T_cAF operated at a lower airflow rate (5600 m^3/h) than LAF, but higher than TMA (3200 m^3/h) (Figure 2e—f and 2a—b, respectively). A technical inspection of the ventilation performance of three systems was carried out before the study commenced to ensure that they functioned as intended.

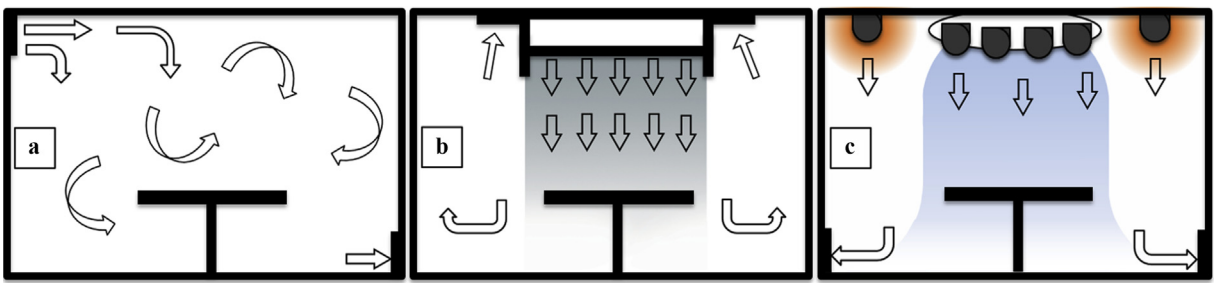

Figure 1. Schematic figures showing the airflow principles of the three ventilation systems: (a) turbulent mixing airflow; (b) laminar airflow; and (c) temperature-controlled airflow.

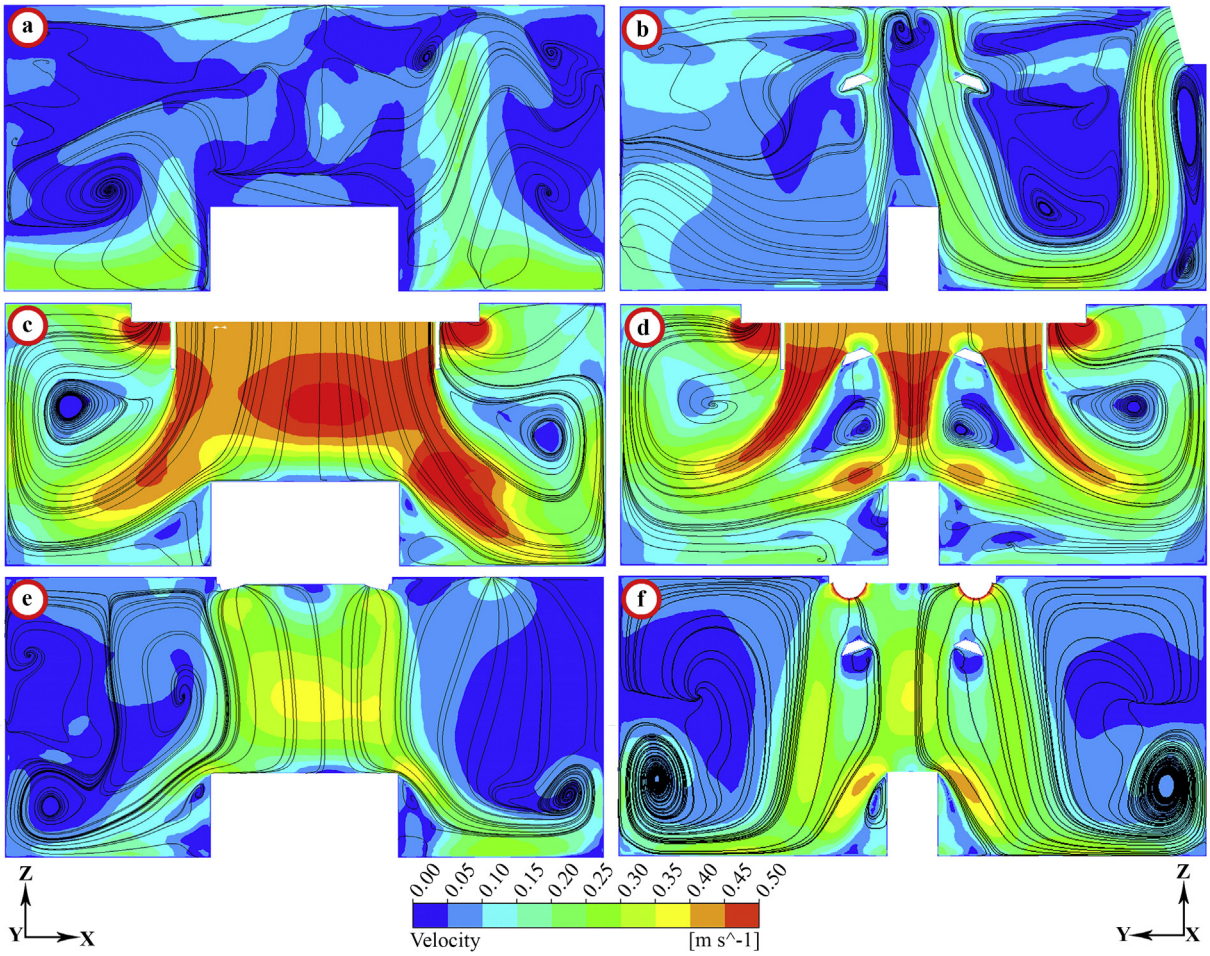

Figure 2. Computational fluid dynamic simulations showing the airflow velocities of the three ventilation systems. The colours in the scale bar represent the different airflow velocities in m/s. The images in the left column are cross-sections of the operating room along the long side of the operating table, and the images in the right column are cross-sections of the operating room along the short side of the operating table. (a,b) Turbulent mixed airflow. (c,d) Laminar airflow. (e,f) Temperature-controlled airflow.

Turbulent mixed airflow

TMA is based on the dilution principle: the airflow is introduced through a high-efficiency particulate air (HEPA) filter to dilute the contamination to a lower level. This entails an exponential decay of high concentrations of airborne microbes over time. Due to turbulent mixing, the concentration will be quite uniform in the entire OR. In this study, the air entered through a panel along the top of a wall in the OR with TMA, and exited close to the floor in the corners of the opposite wall (Figure 1a).

Laminar airflow

LAF ventilation, more correctly called 'unidirectional airflow', pushes the air through HEPA filters in the ceiling above the operating table at a high airflow rate so that a vertical speed of 0.4 m/s is achieved (Figure 2c–d). The airflow speed should be high enough to preserve its unidirectional flow even when disturbed by personnel and equipment, but low enough to hinder turbulence. The incoming air entered the room from a 2.75 × 2.75 m^2 box above the operating table, creating an ultra-clean zone below the box, and exited through the ceiling just outside the box (Figure 1b).

Temperature-controlled airflow

The newly developed T_cAF ventilation system uses cooled HEPA-filtered air above the operating table that flows downwards due to higher density than the surrounding air which is 1.5°C warmer. The cooled and filtered inlet air is introduced from eight half-spherically shaped air diffusers mounted in a circle, creating an ultra-clean zone that expands from the centre of the room (Figure 1c). Surrounding the cooled central airflow, warmer HEPA-filtered air is dispersed from eight additional ceiling-mounted half-spherically shaped air diffusers. The warm air prevents stagnation zones in the periphery of the room and maintains the temperature gradient that drives the central vertical flow of cooled air. Thus, the temperature gradient of 1.5°C is maintained by controlling both the cooled central air and the surrounding warm air. The airflows are high enough (>0.25 m/s) to counteract body convection from staff around the operating table, and heat convection from lamps and other equipment.

Sampling and quantification of cultivable airborne bacteria

Airborne bacteria were measured at three locations: close to the wound, at the instrument table and in the periphery of the room. The cfu count was used as a measure of viable airborne bacterial loads. The cfu concentration, at a distance of ≤40 cm from the wound, was determined by air sampling on vertically oriented 80-mm-diameter gelatine filters (MD8 airscan, Sartorius GmbH, Göttingen, Germany). Immediately after sampling, the filter was transferred to a blood agar culture medium. The cfu concentrations at the instrument table and in the periphery of the room were measured by direct impaction of airborne cells on blood agar Petri dishes with rotating slit samplers (Impactor FH5, Klotz GmbH, Bad Liebenzell, Germany). No tubing was used at the inlets of the rotating slit samplers as this results in lower cfu counts due to particle losses. The samplers were cleaned with DAX 45% ethanol surface disinfectant before surgery commenced. For both the filter sampler and the slit samplers, the sampling time was 10 min with an airflow rate of 100 L/min. Measurements started at wound incision and ended at wound closure. All Petri dishes were incubated for 48 h at 35°C, and the cfu were subsequently counted and divided into major species by the Clinical Bacteriological Laboratory at the University Hospital, Lund, Sweden. During cfu sampling, observations were made of the number of staff present and the number of door openings in the ORs. The measurements from three surgeries (one in TMA and two in T_cAF) were discarded because of deviations from the protocol; additional surgeries were thus performed to reach the 15 surgeries in each OR. Due to condensed water on the lid and contamination, 12 cfu plates from different surgeries (11 in TMA and one in LAF) were not valid to analyse. A comparison of methods for measuring airborne microbial particle concentration in ORs was performed using a slit sampler and a real-time viable particle counter (BioTrak, TSI Inc., Shoreview, MN, USA). The BioTrak utilizes autofluorescence from biomolecules (NADH and riboflavin) to discriminate between microbial and non-microbial particles in the air, and applies an algorithm, based on experimental data of airborne micro-organisms, to identify viable particles.

Working environment survey

A working environment survey evaluated how the operating staff experienced the impact of ventilation. They answered a questionnaire with six questions concerning temperature, draught, noise and perceived comfort after finishing an operation. The questions and more details are provided in Appendix A.

Statistical analysis

The non-parametric Mann-Whitney *U*-test was used to investigate pairwise differences between the ORs. The Sign test was used to compare the median cfu concentrations with the recommended limit of 10 cfu/m^3. The correlation between cfu count and selected factors, such as door openings and number of people present, was tested using non-parametric Spearman rank-order correlation test. *P*-values <0.05 were considered to indicate significance. Statistical analyses were performed using SPSS Version 23 (IBM Corp., Armonk, NY, USA).

Results

Concentrations of cultivable bacteria in ORs during ongoing surgery

In this study, 750 air samples for cfu concentration measurements from 45 surgeries (on average, six samples per location and surgery) were collected and analysed. The measurements showed that both LAF and T_cAF had a median cfu concentration below 10 cfu/m^3 at all locations in the room during ongoing surgery (Figure 3, Table I). However, the median cfu concentration for LAF in the periphery of the room was not significantly below 10 cfu/m^3 ($P = 0.28$). TMA had cfu concentrations equal to or above 10 cfu/m^3 at all locations during surgery.

The three ventilation systems resulted in substantially different cfu concentrations. At the wound and the instrument table, both located inside the ultra-clean zone in LAF and T_cAF, the lowest cfu concentrations were found in LAF (median 0 cfu/m^3). T_cAF had higher cfu concentrations at these locations (median 1–3 cfu/m^3), and TMA had even higher cfu concentrations (median 10–22 cfu/m^3). As expected, TMA had similar cfu concentrations at all locations in the room, and the concentrations at the three locations were positively correlated. For LAF and T_cAF, the cfu concentrations correlated between the wound and the instrument table, but not with the periphery of the room. As described in Appendix B, the slit sampler provided higher cfu values than the filter sampler ($P < 0.05$), and thus the lower cfu values close to the wound are partly a result of measurement methodology. Approximately equal amounts of *Micrococcus* spp. and *Staphylococcus* spp. dominated in all ventilations, with a small fraction of *Bacillus* spp. (2%) (Appendix C, Table C.I). No correlation was found between cfu/m^3 and viable particle count by fluorescence (BioTrak) (Appendix D, Figure D.1).

The cfu concentrations in the different ventilation systems cannot be explained entirely by the differences in airflow rates. This is shown by the comparison of the measured cfu concentrations and the general assumption that the cfu concentration is inversely proportional to the airflow rate (Figure 4). For instance, T_cAF only had twice the airflow of TMA, but one-tenth of the cfu concentration at the wound (Figure 3, Table I). Similarly, LAF had four times higher airflow than TMA, but the cfu concentration was less than one-twentieth of that at the wound. Thus, T_cAF and LAF use the airflow more efficiently than TMA to remove bacteria. Consequently, the higher energy consumption by LAF and T_cAF were small compared with the lowering of cfu concentration: T_cAF and LAF used two and three times more energy than TMA, respectively (Table I).

Effect of door openings on cfu concentrations

No significant correlation was found between the total number of door openings per surgery (normalized by duration of surgery) and the average cfu concentration at the wound (Appendix E, Figure E.1). The adjacent preparation room and

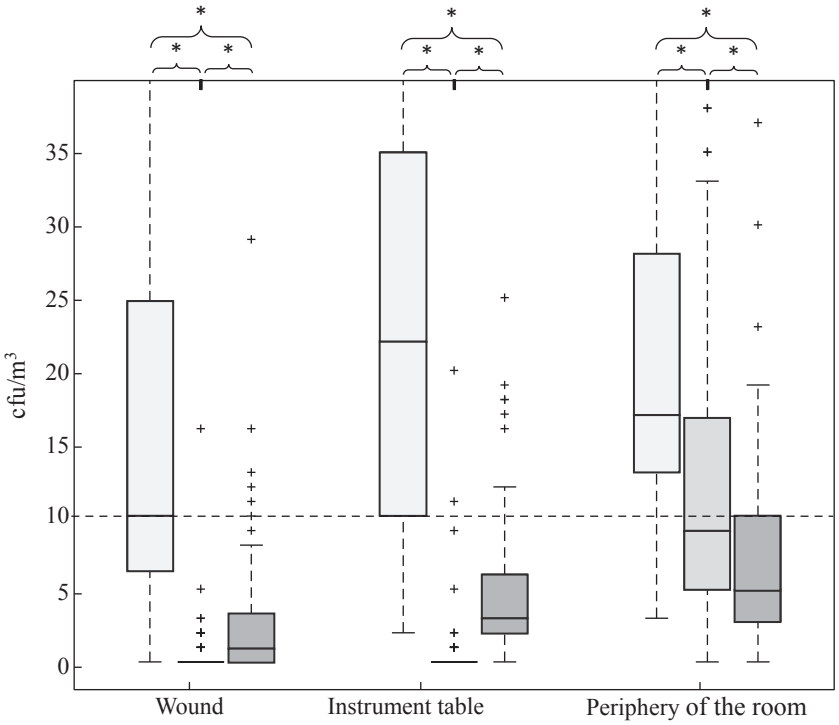

Figure 3. Box plot showing the median (middle line in box) colony-forming units (cfu)/m^3 at the wound, instrument table and in the periphery of the room for three different ventilation systems: turbulent mixed airflow (TMA, white bars), laminar airflow (LAF, light grey bars) and temperature-controlled airflow (T_cAF, dark grey bars). The dashed line at 10 cfu/m^3 is the limit for ultra-clean air [1]. Groups at each measuring location are all significantly different (* indicates $P < 0.01$).

the exit room were both efficiently ventilated, and consequently, only door openings to the corridor were included. In the corridor, the median concentration was 40 cfu/m^3 ($N = 22$, range 18–66 cfu/m^3). The median number of door openings to the corridor per surgery was highest in LAF and lowest in T_cAF (Table I). Other parameters that were investigated included the number of people present in the room, which had low variation (Table I), and the duration of surgery (median 70 min, range 40–120 min), but no significant correlation was found in either case.

Impact of ventilation system on the working environment

The analysis of the survey responses of the operating personnel (response frequency: TMA = 25, LAF = 28 and T_cAF = 29) indicated that LAF was experienced as noisy and causing a noticeable draught, while T_cAF and TMA were perceived as creating a more comfortable working environment (Table A.I, Figure A.1). Values on the noise level

Table I
Ventilation system parameters and colony-forming unit (cfu) values for turbulent mixed airflow (TMA), laminar airflow (LAF) and temperature-controlled airflow (T_cAF). The ventilation power values are based on calculations made by an external consulting agency

Ventilation	TMA	LAF	T_cAF
Airflow (m^3/h)	3200	12,000	5600
Recirculation of air (%)	0	70	45
Noise in empty room (dBA)	45	58	48
Ventilation power (kW)	2.8	8.0	5.7
cfu/m^3, median (range, number of samples)			
Wound	10 (0–162, 71)	0 (0–16, 90)	1 (0–29, 81)
Instrument table	22 (2–100, 82)	0 (0–20, 91)	3 (0–25, 81)
Periphery of the room	17 (3–90, 82)	9 (0–38, 91)	5 (0–37, 81)
Observation of activities, median (range)			
Door openings per surgery (normalized to number/h)	5.6 (0–11)[a]	3.8 (1.4–11)[a]	2.1 (0–10)[a]
Number of people in the room	7 (6–8)[a]	7 (5–9)[a]	7 (6–9)[a]

[a] Differences between the groups were not significant using Kruskal–Wallis test ($P = 0.06$).

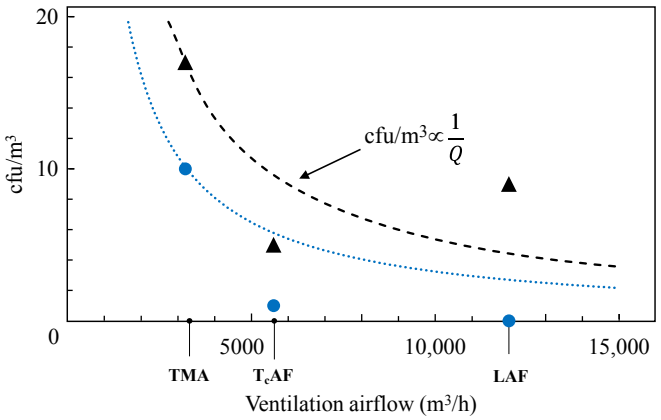

Figure 4. Median values of colony-forming unit (cfu) concentrations at the wound (marked ●) and in the periphery of the room (marked ▲) in relation to the ventilation airflow rate. The dashed and dotted lines represent the assumption that the cfu count/m^3 is inversely proportional to the airflow rate (cfu/m^3 ∝ 1/Q), where Q is the airflow rate in m^3/s. Both curves are adjusted to the median values for turbulent mixed airflow (TMA). The dotted (blue) line corresponds to the wound concentrations and the dashed (black) line corresponds to the concentrations in the periphery of the room. The airflow rates for the ventilation systems are 3200 m^3/h for TMA, 5600 m^3/h for temperature-controlled airflow (T_cAF) and 12,000 m^3/h for laminar airflow (LAF).

measured in empty ORs (Table I) were directly related to reported noise disturbances. The complete survey questions and answers are provided in Appendix A.

Discussion

Ultra-clean air, defined as air with less than 10 cfu/m^3, is suggested for implant surgery and infection-prone surgery to minimize SSI [1]. This study found that LAF and T_cAF provided air cleanliness below this limit in the entire OR during surgery. The cfu concentrations in TMA were higher than the recommended limit at all positions in the OR, which compromises its usage for infection-sensitive surgery. Differences in airflow rates for the three ventilation techniques influenced the cfu concentrations, but these do not fully explain the differences between the cfu values obtained. T_cAF and LAF had airflow rates that were two and four times higher than for TMA, but provided cfu concentrations that were four to 20 times lower, with the exception of the peripheral room location in LAF. Hence, it is clear that both airflow rate and direction of airflow influence the cfu concentrations for the ventilation techniques, and that T_cAF and LAF are more efficient in removing bacteria than TMA. By directing the clean incoming airflows strategically, there is potential to lower the airflow rates, leading to energy savings that are beneficial for both the hospital's economy and the environment. Another possible advantage of using lower airflows in the OR is that this may reduce the cooling effect and thus decrease the risk for patient hypothermia, which has been shown to be a risk factor for SSI [15].

The cfu measurement results showed substantial variations during and between operations, and neither the number of door openings nor number of people present during surgery correlated with cfu concentrations. The large variations could be due to individual variations in microbial particle shedding and the degree of activity, which was also found in an earlier study [10]. The overall low cfu levels in the present study, compared with, for example, the results by Agodi *et al.* [14], could be explained by meticulous hygiene routines and staff awareness. Consequently, a temporary lack of compliance with hygiene routines may be one reason for high cfu values and large variations between surgeries. Similar to several earlier studies [5,10,12,16], no correlation between the number of people in the OR and cfu concentrations was observed in this study. One hypothesis is that cfu concentrations are correlated to the activity of the staff rather than the actual number of people present [10]. Model calculations have shown, however, that there is still reason to have fewer people present during surgery, as the airflow pattern may be disrupted, causing enhanced risk for contamination [17]. Most studies have shown significant correlation between cfu concentrations and door openings [10–12,14,16]. In the present study, no significant relation was found. This is explained by a low number of door openings and low cfu concentrations in the corridor outside the OR.

One limitation of this study is that many designs exist for both TMA and LAF ventilations. For instance, there are TMA systems with higher airflows, and LAF systems with lower airflows. The LAF ventilation studied was designed as a Charnley box, with short curtains; however, there are many other versions where outlets are positioned differently (partly to reduce noise), or where temperature gradients are used. Despite this, the ventilation systems investigated produce characteristic flow patterns for each type, and thus the measurements are representative and meaningful for comparison. This study was executed in three identical ORs with staff from the same operating unit with the same routines and resources. No follow-up on SSI from these surgeries was undertaken as 100 times more operations would be needed to see any statistical differences.

Using cfu as a measure of airborne microbial load, which is a common standard in hospital hygiene, can be questioned as only a small fraction of all bacteria are cultivable [18]. Thus, there is a risk that bacterial cells that are viable and potentially infectious, but unable to grow on nutrition plates, will be missed by cultivation techniques (cfu). Clarke *et al.* [19] used polymerase chain reaction (PCR), which is a molecular-cultivation-independent technique, to assess the presence of specific DNA sequences in the environment, and compared the results with cfu counts for analysing tissue samples. In several cases, no bacteria were found using cfu counts, while the PCR analysis gave positive results. However, PCR does not provide information on viability, and thus should be used as a complement to other methods. In the present study, the comparison of a real-time viable particle counter (BioTrak) and cfu/m^3 showed no correlation (Figure D.1), yet it was clear that the BioTrak measured substantially higher concentrations (range 0–544 viable particle counts/m^3) of bacterial particles in the air.

Regardless of a considerable variation in the efficiency of different sampling techniques, there are no precise standards on how air cleanliness measurements should be performed. Active air samplers were used in this study to obtain volume concentrations. The filter sampler could be operated with a sterile hose, enabling sampling close to the wound without disturbing the sterile environment at the operating table. The

slit sampler was considerably easier to handle and also less noisy, and were thus used at the instrument table and in the periphery of the room. The comparison of collection efficiency for the air samplers (Figure B.1) showed that the filter sampler measured significantly lower cfu concentrations than the slit sampler, which could be explained by different sampling orientations and desiccation during filter sampling leading to reduced viability of bacteria [20].

The working environment is critical for medical staff in ORs as they are expected to perform advanced tasks that demand high attention. Ventilation systems with high air exchange rates are often noisy and may create a cold draught that can cause tension in the shoulders, which was reflected in the answers from the working environment questionnaire. Thus, one important aspect of the ventilation system is its impact on the working environment. Obtaining low cfu values ($<10\,cfu/m^3$) without special garments may be of importance for the staff's working environment. With an impending threat of antibiotic-resistant bacteria in hospitals, it may not be possible to choose between ultra-clean ventilation and occlusive clothing in the future, which makes permanent installations, such as the ventilation system, a keystone in infection prevention efforts.

In conclusion, comparison of three ventilation systems in three identical ORs showed that LAF and T_cAF provide the high air cleanliness that is needed to perform infection-prone surgery ($<10\,cfu/m^3$). TMA has cfu levels that are too high to be classified as an ultra-clean ventilation system at all locations in the OR (i.e. $<10\,cfu/m^3$). The lower performance of TMA can only partly be attributed to a lower airflow. The working environment and energy consumption are often neglected in evaluations of ventilation for operating systems. Nevertheless, these parameters play an important role for the economy of a hospital and for staff well-being and performance. This study shows that the new T_cAF technology is both more energy efficient and comfortable to work in than LAF, but still provides high air cleanliness.

Acknowledgements

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Conflict of interest statement
Peter Ekolind is the CEO of Avidicare AB, the company that developed temperature-controlled airflow. All other authors report no conflicts of interest relevant to this article.

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Appendix A. Working environment survey

Method

A working environment survey was performed to evaluate the experienced impact of ventilation on the operating staff. Staff members were asked to answer a questionnaire with six questions concerning temperature, draught, noise and perceived comfort after having finished an operation. Each question was answered by marking a number on a scale from 1 to 7, where 1 corresponded to very low/very bad, and 7 was very high/very good.

Questions

1. a. Do you perceive a cold draught from the ventilation in the room?
   1 = Not at all, 7 = Very much
   b. If you perceived a cold draught, how does it affect you?
   Five choices: 1 = Cold shoulders/neck, 2 = Hurts shoulders/neck, 3 = Cold hands, 4 = Headache, 5 = Other (possibility to specify)
2. How do you perceive the temperature control in the room?
   1 = Very bad, 7 = Very good
3. What is your perception of the noise from the ventilation?
   1 = Very silent, 7 = Very loud
4. What is your overall perception of the ventilation in the operating room?
   1 = Very bad, 7 = Very good
5. How does the ventilation affect you?
   Three choices: Positive, Negative, Neither
   If you feel that you are affected, how much would you estimate it to be?
   1 = Very little, 7 = Very much
6. How do you perceive the overall working environment comfort in the room?
   1 = Very bad, 7 = Very good

Results

Table A.I
Results from the working environment survey in laminar airflow (LAF), turbulent mixed airflow (TMA) and temperature-controlled airflow (T_cAF)

Question	LAF	TMA	T_cAF
1.a	4	3	2
1.b (freq.)	12,3,3,2,1	11,1,6,0,0	11,1,7,0,0
2.	3	4	5
3.	7	2	2
4.	3.5	4	6
5. Negative (*N*)	5 (19)	5 (3)	5 (2)
5. Positive (*N*)	2 (1)	1 (1)	4 (11)
6.	3	4	5

The median is reported for the questions that were answered with scores of 1–7. For Question 1.b, the frequencies for each choice are reported. The answers for Question 5 are separated into two medians: one for being positively affected and one for being negatively affected. Here, the number of respondents is given in parentheses. The total response frequency was: TMA = 25, LAF = 28, T_cAF = 29.

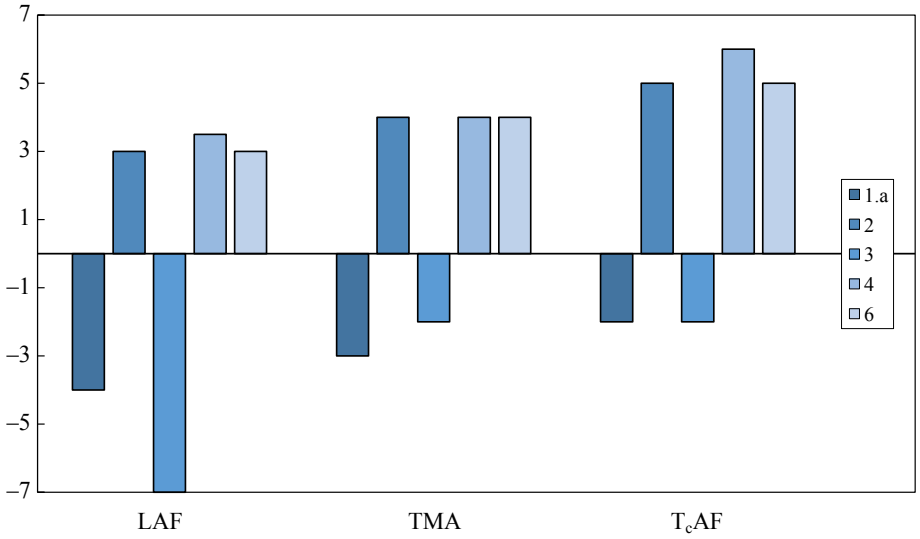

Figure A.1. Results from the working environment survey with the median values for Questions 1.a, 2, 3, 4 and 6, where the answers from Questions 1.a and 3 are reported as negative and the answers from Questions 2, 4 and 6 are reported as positive. A negative rating is given to questions where a high score is equivalent to a bad working environment. LAF, laminar airflow; TMA, turbulent mixed airflow; T_cAF, temperature-controlled airflow.

Appendix B. Comparison of Sartorius filter sampler and Klotz slit sampler

Introduction and method

The instruments used for cfu concentration measurements have two different sampling principles: impaction and filter collection. In the slit sampler, bacteria receive immediate nutrition once they impact on the agar plate. Bacteria collected on gel filters reach nutrition when the runtime is over and the filter is dissolved on an agar plate. The slit sampler has a cut-off diameter around 3–4 μm and thus a poor sampling efficiency of particles below this size. Consequently, the filter sampler has a higher physical collection efficiency than the slit sampler. To investigate if there was a difference in sampling efficiency, the cfu counts from the slit sampler and the filter sampler were compared by parallel measurements in a hospital corridor. No tubes were used at the inlets of the air samplers to ensure no particle loss.

Results and discussion

In the hospital corridor, the two parallel air samplers collected 44 air samples (see Figure B.1). The median cfu concentration measured by the slit sampler was 40 cfu/m^3 (range 18–66 cfu/m^3), and the median cfu concentration measured by the filter sampler was 16 cfu/m^3 (range 0–80 cfu/m^3). The collecting agar plate in the slit sampler was positioned horizontally, whereas the filter sampler was oriented vertically. The difference in orientation may be a reason why the slit sampler values are higher, since μm-sized particles settle due to gravitation and are hence easier to collect on a horizontal surface than on a vertical surface. Another reason is that bacteria are exposed to desiccation on the filter throughout the collection time, which may decrease viability and reproducibility. It has been shown that sensitive bacteria (e.g. *Escherichia coli*) are negatively affected by dehydration when collected by filter sampler [20].

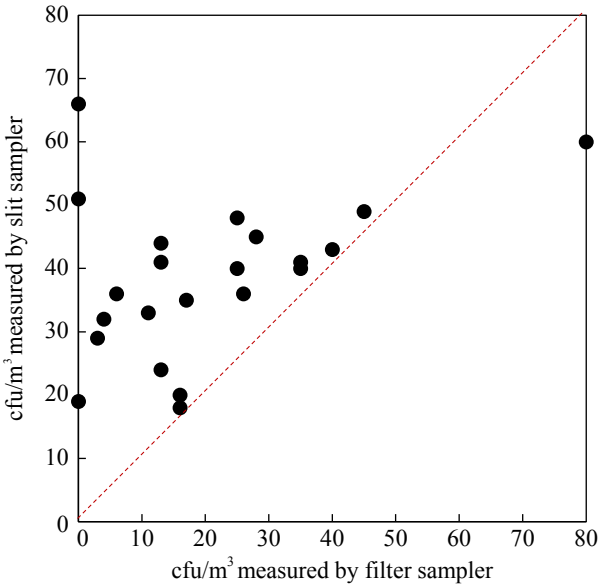

Figure B.1. Comparison of colony-forming unit (cfu) concentrations measured by slit sampler and filter sampler (N = 22). The dashed (red) line represents the 1:1 ratio.

Appendix C. Classification of colony-forming unit bacteria genera

Results

The cfu counts from three major bacterial genera, which are easily recognized by their appearance on agar plates, were

documented: *Staphylococcus* spp., *Micrococcus* spp. and *Bacillus* spp. On average, these genera represented 42%, 57% and 2% of all cultivable bacteria, respectively, regardless of the ventilation system. TMA showed the highest proportion of *Micrococcus* spp., while LAF had a higher proportion of *Staphylococcus* spp., and T_cAF had similar proportions of both *Staphylococcus* spp. and *Micrococcus* spp. (see Table C.I). The proportion of *Bacillus* spp. was low (2%) in all ventilation systems. In addition to the results presented in Table C.I, the presence of the following genera were detected at single events: *Moraxella* spp., *Acinetobacter* spp., *Corynebacterium* spp., *Lactobacillus* spp. and mould.

Table C.I
Percentages of the three bacteria genera included in the study, presented for each of the ventilation systems, all three measurement locations included

	% *Staphylococcus* spp.	% *Micrococcus* spp.	% *Bacillus* spp.
TMA	36 (10)	62 (14)	2 (0)
LAF	59 (3)	39 (1)	2 (0)
T_cAF	51 (9)	47 (8)	2 (1)

LAF, laminar airflow; TMA, turbulent mixed airflow; T_cAF, temperature-controlled airflow.
The numbers in parentheses are the percentages of each genera at the wound location alone.

Appendix D. Comparison of colony-forming unit measurements with fluorescent viable counts by the Biotrak

Method

A comparison of methods for determining the amount of airborne microbial particle concentration was performed using cfu concentrations measured by a slit sampler and viable particle concentration measured by BioTrak. The two air samplers were positioned side by side and measured in parallel during surgery in the ORs. Measurements were taken during orthopaedic surgeries in the ORs with LAF and T_cAF ventilation.

Results

No correlation was found between the two methods, which could be expected since they use totally different measures. However, it was clear that the BioTrak measures higher values of airborne bacteria than the slit sampler (see Figure D.1), with median values of 113 viable particles/m^3 compared with 3 cfu/m^3.

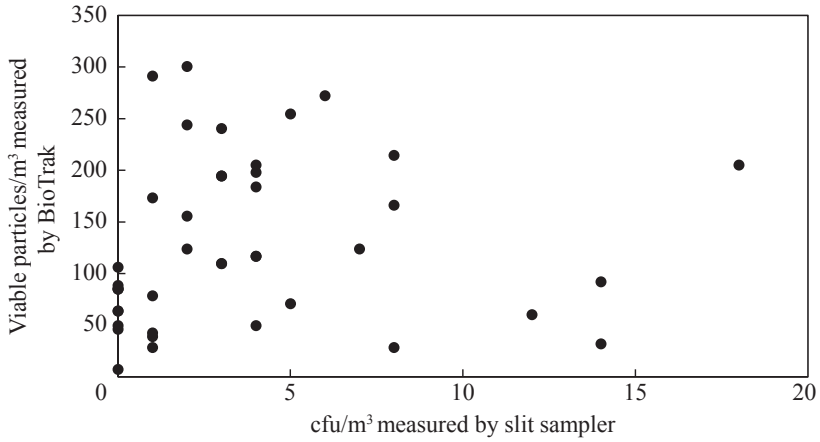

Figure D.1. Comparison of colony-forming unit (cfu) concentrations measured by the slit sampler with viable particle concentration measured by BioTrak (using autofluorescence from biomolecules). One sample point was omitted from the plot due to high values (40, 544).

Appendix E. Door openings correlated to colony-forming units alone in laminar airflow (LAF) ventilation

Results

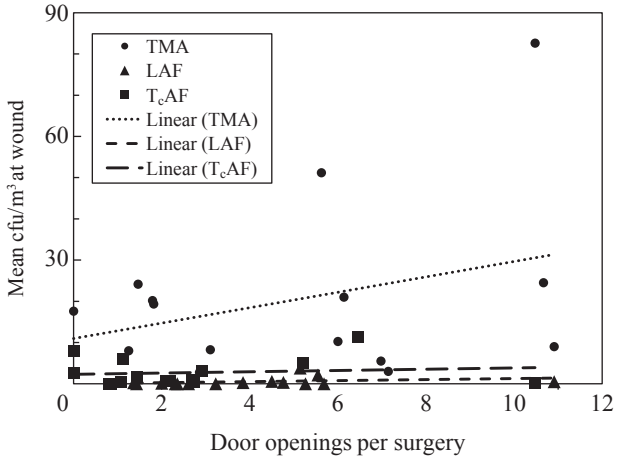

Figure E.1. Average colony-forming units (cfu) at wound location in relation to the number of door openings during surgery. The number of door openings is divided by duration of surgery. The R^2 values for the fitted lines are 0.106 for turbulent mixed airflow (TMA), 0.081 for LAF and 0.017 for temperature-controlled airflow (T_cAF).

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