Operating Room Air May Harbor Pathogens: The Role of An Ultraviolet Air Filtration Unit.

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Abstract

Prevention of surgical site infections involves implementation of numerous steps including ultraclean air in the operating room (OR). We conducted this prospective study to determine and compare the nature and quantity of microbes in the OR, as detected from the inlet and outlet flow of an ultraviolet filtration unit. To do so, a filtration unit with a crystalline ultraviolet unit (C-UVC) was placed in the OR. The inflow and outflow air from the unit was sampled at the beginning and at conclusion of each surgical procedure. We recorded surgical-related variables and processed the air swabs for culture and Next-Generation Sequencing. The mean length of the surgical procedures sampled was 68±13 minutes. Overall, 19 out of 200 (9.5%) swabs isolated microorganisms. Inflow swabs were positive at a higher rate (16% vs. 3%; p<0.01), compared to the outflow air swabs. A wide variety of Gram-positive, Gram-negative, anaerobic bacteria, and fungi were isolated. The detection of microorganisms was higher in light of a higher number of door openings (32.5±7.1 vs. 27.9±5.6; p<0.01). Our study showed that microorganisms are present in the OR. Moreover, a specialized filtration unit with a C-UVC light was effective in filtering these microorganisms in the majority of cases.

INTRODCUTION

The air in the operating room (OR) can act as an important vehicle for transfer of pathogens to the surgical site[1–3]. Previous studies have demonstrated a clear correlation between the amount and size of airborne particles and the detection of viable floating microorganisms[4,5]. In fact, these studies have also pointed out the link between Colony Forming Units (CFUs) in the OR environment and a higher incidence of surgical site infections (SSIs)[2,6,7]. Thus, one of the important factors for prevention of SSIs is ultraclean air in the OR[8,9]. Even so, it is recognized that the OR cannot be made particle free[9].

Different technologies and strategies have emerged to minimize the overall number of particles, including pathogens, in the OR[10]. Positive-pressure ventilation systems and unidirectional laminar flows (LAF) are commonly employed[7,8,10,11]. Nevertheless, even though these ventilation systems may posit an advantage towards a cleaner air environment, the reality is that the presence of physical barriers, such as equipment, personnel traffic, door openings, and heat sources within the OR may interfere with the flow[10,12,13]. Moreover, positive-pressure ventilation may only be achieved in a restricted surgical area[10].

Previous studies have explored the role of crystalline ultraviolet-C (C-UVC) filtration units in the OR and demonstrated a benefit for the use of such units[12,14,15]. These units can filter and deactivate airborne particles, some of which are known pathogens[3,14,16]. The air flow enters at the bottom of the unit and passes through a copper and carbon cartridge. Then, the air is directed into a chamber with C-UVC light that inactivates pathogens[14,17]. After that, air passes through a high efficiency particulate air (HEPA) filter and flows out at the top of the unit[14]. The additional use of this unit was shown to reduce the total and viable particle counts[12,15] in the OR by 65%[15].

This prospective study aimed to determine and compare the nature and quantity of microbes in the operating room, as detected from the inlet flow of an ultraviolet filtration unit, and the efficacy of the unit to remove particles and creating clean room air (the outlet flow).

RESULTS

Inflow and outflow air swabs

Overall, 19 out of 200 (9.5%) swabs isolated microorganisms. Of these, 19 (9.5%) were positive for culture and 4 (2%) also identified microorganisms by NGS. *Staphylococcus* spp were the most frequent genre of bacteria isolated (16/19, 84.21%). The 4 swabs that were simultaneously positive for culture and NGS isolated Gram-positive, Gram-negative, anaerobic bacteria, and fungi (Table 1).

Table 1
Results of 200 air swabs, by culture and NGS analysis.

Samples	Culture	NGS	Culture and NGS
Inflow Air (n = 100)	**Positive (n = 16 [16%])**	**Positive (n = 3 [3%])**	**Positive (n = 3 [3%])**
	Staphylococcus haemolyticus (n = 6)	*Staphylococcus epidermidis (n = 2)*	*Staphylococcus epidermidis (n = 2)*
	Staphylococcus epidermidis (n = 4)	*Staphylococcus haemolyticus (n = 1)*	*Staphylococcus haemolyticus (n = 1)*
	Staphylococcus hominis (n = 2)	*Staphylococcus hominis (n = 2)*	*Staphylococcus hominis (n = 2)*
	Staphylococcus salivarius (n = 1)	*Staphylococcus cohnii (n = 2)*	*Staphylococcus cohnii (n = 2)*
	Staphylococcus cohnii (n = 1)	*Corynebacterium amycolatum (n = 1)*	*Corynebacterium amycolatum (n = 1)*
	Staphylococcus capitis (n = 1)	*Corynebacterium tuberculostearicum (n = 1)*	*Corynebacterium tuberculostearicum (n = 1)*
	Corynebacterium afermentans (n = 3)	*Lactobacillus crispatus (n = 1)*	*Lactobacillus crispatus (n = 1)*
	Moraxella osloensis (n = 1)	*Pseudomonas luteola (n = 2)*	*Pseudomonas luteola (n = 2)*
	Mycobacterium neoaurum (n = 2)	*Propionibacterium namnetense (n = 1)*	*Propionibacterium namnetense (n = 1)*
	Pantoea spp (n = 2)	*Moraxella osloensis (n = 2)*	*Moraxella osloensis (n = 2)*
	Janibacter spp (n = 1)	*Listeria monocytogenes (n = 2)*	*Listeria monocytogenes (n = 2)*
	Jeotgalicoccus spp (n = 1)	*Pantoea spp. (n = 3)*	*Pantoea spp. (n = 3)*
	Cutibacterium acnes (n = 1)	*Cutibacterium acnes (n = 1)*	*Cutibacterium acnes (n = 1)*
		Finegoldia magna (n = 1)	*Finegoldia magna (n = 1)*
		Rhodotorula mucilaginosa (n = 2)	*Rhodotorula mucilaginosa (n = 2)*
		Cladosporium colombiae (n = 1)	*Cladosporium colombiae (n = 1)*

Samples	Culture	NGS	Culture and NGS
Outflow air (n = 100)	**Positive (n = 3 [3%])** *Staphylococcus epidermidis* (n = 1) *Citrococcus* spp (n = 1) *Cutibacterium acnes* (n = 1)	**Positive (n = 1 [1%])** *Staphylococcus epidermidis* (n = 1) *Staphylooccus haemolyticus* (n = 1) *Pseudomonas luteola (n = 1)* *Cutibacterium acnes* (n = 1) *Finelgoldia magna* (n = 1)	**Positive (n = 1 [1%])** *Staphylococcus epidermidis* (n = 1) *Staphylooccus haemolyticus* (n = 1) *Pseudomonas luteola (n = 1)* *Cutibacterium acnes* (n = 1) *Finelgoldia magna* (n = 1)

Among the 100 swabs sampling the inflow air to the Illuvia Sense unit, 16 (16%) isolated organisms. Of these, 3 (3) % had a simultaneous isolation by NGS. A wide variety of Gram-positive, Gram-negative, and anaerobic bacteria were isolated from the inflow air swabs. A mold and yeast species were also isolated in the inflow air swabs. By contrast, the culture of outflow air swabs was positive at a lower rate (3%; $p < 0.01$), isolating *Staphylococcus* species and anaerobes. Additional microorganisms, e.g., *Pseudomonas luteola*, were also isolated by NGS in 1 (1%) outflow air swab.

Surgical-related variables

The mean length of the surgical procedures, from surgical incision to application of dressing, sampled was 68 ± 13 minutes (Table 2). Moreover, a mean of 8.7 ± 1.2 surgical personnel were found in the OR and a mean of 28.3 ± 5.9 door openings occurred in each case. We detected a mean count of 3.845 ± 1.933 particles of 5 µm/cm^3 at the time of swabbing. The mean CO_2 level and temperature was 559.12 ± 24.84 parts *per* million (PPM) and 65.29 ± 1.37°F, respectively. The analyses demonstrated that swabs were more likely to be positive in cases with more door openings (32.5 ± 7.1 vs. 27.9 ± 5.6; $p < 0.01$). The latter applied to particles at 0.5 µm (4.959 ± 1.956 vs. 3.730 ± 1.899, $p < 0.01$), 1 µm (0.863 ± 0.288 vs. 0.641 ± 0.329, $p < 0.01$), and 2.5 µm (0.022 ± 0.007 vs. 0.016 ± 0.009, $p < 0.01$).

Table 2
Surgical-related variables collected at air swab sampling.

	Overall n = 200	Microbial positive air swab n = 19 (9.5%)	Microbial negative air swab n = 181 (90.5%)	p-value
Length (min)	68 ± 13	71 ± 13	68 ± 13	0.33
Personnel #	8.71 ± 1.28	9.26 ± 1.41	8.65 ± 1.25	0.61
Door openings #	28.35 ± 5.9	32.58 ± 7.11	27.92 ± 5.61	< 0.01
0.5 µm particles/cm^3	3.845 ± 1.933	4.959 ± 1.956	3.730 ± 1.899	< 0.01
1 µm particles/cm^3	0.661 ± 0.331	0.863 ± 0.288	0.641 ± 0.329	< 0.01
2.5 µm particles/cm^3	0.017 ± 0.009	0.022 ± 0.007	0.016 ± 0.009	< 0.01
4 µm particles/cm^3	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.001	> 0.99
CO_2 level (PPM)	559.12 ± 24.84	559.58 ± 28.41	559.07 ± 24.53	0.93
Temperature (° F)	65.29 ± 1.37	65.02 ± 1.52	65.32 ± 1.35	0.36

DISCUSSION

Several strategies have been utilized in order to achieve a cleaner operating room (OR) air[1,10,11,15]. The use of positive-pressure ventilation system, with or without laminar flow, is one such strategy [10,11]. Despite, the use of ventilation systems and depending on the number of door openings[1], particles in the air of OR are still present[9].

Our study demonstrated that microorganisms, some being recognized pathogens, are present in the OR room air. The swabs were positive in 9.5% of cases. This finding corroborates the previous findings of Hijji et al., that found a contamination rate of 13.3% at 60 minutes[15]. Variations in contamination rates may be the result of different baseline air ventilation systems installed, their integrity, type of surgery, and methodology employed for sampling[18].

Laminar air flow (LAF) systems are also utilized to reduce the number of particles in the OR room air. The LAF functions by blowing unidirectional flow of air with a known velocity and pressure (> 15 Pa) inside an enclosed area[10]. Under LAF ventilation systems, HEPA filters are also commonly employed[10], which are

claimed to remove more than 99.97% of particles ≥ 0.3 μm[11,19,20]. However, physical barriers such as equipment or personnel in the OR can interfere with the ideal performance of LAF systems[10]. Because of the latter and high cost associated with installation and maintenance, the medical community has questioned the use of LAF in the OR[18,21].

In recent years, there has been an effort to supplement the ventilation system of the OR with additional technologies such as the filtration units with C-UVC sterilizing light[12,15]. We assessed the Illuvia Sense units in the OR of a single institution that were equipped with positive-ventilation system. This study demonstrated that the microorganisms were mainly detected from the inflow, rather than the outflow, of the Illuvia Sense unit, demonstrating clear efficacy of the units in removing particles and/or microorganisms. The unit functions by having the inflow air passing through a copper and carbon activated filter. The particles in the inflow air are inactivated by a C-UVC light. Previous studies have shown great efficacy for UV light in reducing SSI rates (11.3–0.24%) but the use of such technology necessitated protection and shielding of the personnel, which is not required with Illuvia system [22]. In addition the unit delivers UVC light at a wavelength of 200–280 nm, which is proven to inactivate pathogens without posing an additional health risk when used at appropriate intensities (max. 25–30 μW cm^2)[17,23]. In fact, a recent study demonstrated germicidal effects on most ESKAPE-E (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, *Enterobacter* spp, and *Escherichia coli*[24]) pathogens within 2 seconds of exposure to UVC at an intensity as low as 15.150 μW/cm^2 [25]. In addition, the inactivation by C-UVC light (254 nm) was only executed in the UV chamber inside the Illuvia Sense unit. In addition to C-UVC light, the unit also contains a HEPA filter that further removes particles.

It is interesting, and perhaps worrisome, to note that a wide variety of Gram-positive, Gram-negative, anaerobes, and fungi were detected from the inflow air in some of our cases. *Staphylococcus* spp. was the most frequent, similar to previous reports[26]. These bacteria are well-known causative agents of SSIs worldwide[27]. *Corynebacterium* species were also frequent, followed by anaerobes and Gram-negative bacteria. *Cutibacterium acnes* and *Finegoldia magna* were the principal anaerobic species identified. Both are frequent among culture-negative orthopaedic infections[28], especially from clinical samples of the upper-limbs[29].

The study, like previous studies [1,5,30], confirmed that door opening was associated with an increase in the number of particles or microroganims in the OR room air.. A clinical simulation from our institution previously demonstrated that a positive correlation exists between floating particles in the OR and the total number of personnel and door openings performed[1]. It is not surprising to find a higher particle count in procedures with positive swabs, since pathogens cluster and travel in particles 5 μm or larger, meanwhile smaller particles (≤ 3 μm) usually correlate with tissue combustion[5].

We did not find an association between total PPM and the detection of pathogens; however, the exposition to certain VOCs can cause serious health effects[31,32], as many of these compounds are

carcinogenic[33]. A previous simulation showed that CO_2 concentration can increase up to ~ 300 PPM when 4 people remain inside the OR for 20 minutes[34]. In our study, the concentration of CO_2 reached ~ 559 PPM. Still, our sampling was performed during electrocauterization (a high source of PPM in the OR), our mean number of personnel was higher (~ 8), and the surgical time was tripled (68 minutes). Although we did not take a baseline measurement, this number may have been even higher without the incorporated cartridge in the Illuvia Sense unit. In fact, a clinical simulation showed that the use of a carbon activated filter for 2 hours was able to reduce the number of VOC up to 30%[35]. Further experiments are required to address the full potential of the unit regarding VOC clearance.

Our study also suffers some limitations. First, our control group was comprised by a small, but representative sample of sterile swabs, and air swabs from a standard OR and from a non-sterile corridor. Nevertheless, the main hypothesis of our study did not pursue the comparison between the presence or the absence of the C-UVC filtration unit, as this idea has been addressed before[15]. Second, we were not able to assess our hypothesis without the influence of other variables like the positive-pressure ventilation system[10]. Finally, we only compiled the particle counts at the time of sampling near the Illuvia Sense unit. Our results may not reflect the whole OR environment, since particle concentration is known to vary according to location and operative time[5].

CONCLUSION

Based on the findings of this prospective study, room air fitted with effective positive-pressure ventilation system, appears to contain microorganisms and recognized pathogens. The use of supplementary enclosed C-UVC unit, fitted with HEPA, appeared to reduce the OR room air contamination substantially.

METHODS

Study design

We conducted a prospective study at a single institution, where primary total joint arthroplasty and spine surgeries were performed. The OR was fitted with a positive-pressure ventilation system. Based on previous evidence and following the manufacturer instructions[12,14], filtration units with C-UVC sterilizing light (Illuvia Sense, Aerobiotix Inc, Miamisburg, OH) were placed in the OR at 2 m from the surgical table and 6 m from the main source of air contamination (door to the non-sterile corridor; Fig. 1). The Illuvia Sense unit was started 30 minutes before the surgical incision.

Likewise, additional surgical-related variables were recorded at each time of sampling: number and size of the particles *per* cubic centimeter (cm^3), CO^2 level measured in parts *per* million (PPM) and parts *per* billion (PPB), temperature, door openings, and number of staff within the OR.

Swab and culture protocol

A trained member of the research personnel, while wearing sterile gloves, collected air swabs from the inflow and outflow of the Illuvia Sense unit. Air swabs were obtained from 50 surgeries at the beginning (surgical incision) and at the end (upon application of dressing) of each procedure. The inflow air was sampled by exposing the swabs (ESwab, COPAN™) at 10 centimeters from the prefilter cartridge, located at the right bottom side of the unit. These swabs were waved at a 45° angle to the floor for 3 minutes (Fig. 2). By contrast, the outflow air was sampled by exposing the swabs at 10 centimeters from the HEPA filter, located at the central upper part of the unit. These swabs were waved at a 45° angle to the ceiling.

In total, 4 swabs were collected *per* surgery. The aseptic conditions required for the surgical procedure were not compromised in any case. Two surgical procedures were sampled without starting the Illuvia Sense units and two swabs were exposed for 3 minutes to the non-sterile corridor. These swabs were used as positive controls. In addition, two set of sterile swabs, were processed, and used as negative controls.

All COPAN™ ESwabs were sealed and immediately transported to the laboratory for processing. Each swab was vortexed for 1 minute and 100 uL were inoculated into trypticase soy agar (TSA) and Sabouraud plates. TSA plates were incubated at 37°C for 72 hours under aerobic conditions and at room temperature thereafter. Meanwhile, Sabouraud plates were incubated at 30°C for 14 days. Growth was carefully assessed every 24 hours or until positive. In plates with growth, colonies were counted and plates were sealed and sent overnight for identification by NGS analysis (MicroGen Dx, Lubbock, TX).

Next-Generation Sequencing

All COPAN™ ESwabs and positive plates were shipped overnight for NGS analysis (MicroGen Dx, Lubbock, TX). Each swab was vortexed for 1 minute and processed in ~ 800 uL of the transportation medium. Samples were mechanically lysed using the Qiagen TissueLyser (Qiagen, Hilden, Germany). Then, each sample was spiked with a positive internal control to ensure the success of the extraction process. In addition, negative controls were run along with samples to identify contamination associated with the extraction process. DNA was extracted and then amplified using forward and reverse primers specific to regions flanking the 16S rRNA gene for bacteria and the ITS2 (Internal transcribed spacer) gene for fungi on a LightCycler 480 II (Roche Life Sciences, Indianaoplis, IN, US) with the following thermal cycling profile: 95°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 52°C for 40 seconds, and 72°C for 1 minute; and final extension at 72°C for 10 minutes. Amplified DNA was then be pooled and run on the Illumina MiSeq (Illumina Inc, San Diego, CA).

Statistical Analysis

Descriptive statistics of categorical variables were presented as absolute and relative frequencies. To compare categorical data, we used the Fisher´s exact test or X^2 test, as appropriate. Quantitative variables were assessed for normality using the Shapiro-Wilk test. Then, comparisons were performed with the Student´s *t* test or the Mann-Whitney U test, accordingly. A two-tailed p-value ≤ 0.05 was considered statistically significant throughout analyses.

Declarations

DATA AVAILABILITY

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

J.P. reports personal fees and other from Corentec, personal fees from Data Trace, personal fees from Elsevier, personal fees from Jaypee Publishers, personal fees from SLACK Incorporated, personal fees from Wolters Kluwer, personal fees from Becton Dickensen, personal fees and non-financial support from Zimmer Biomet, personal fees from Ethicon, personal fees from Tenor, personal fees from KCI / 3M (Acelity), personal fees from MicroGenDx, personal fees from Jointstem, personal fees from Becton Dickenson, personal fees from Cardinal Health, other from Parvizi Surgical Innovation and Subsidiaries, other from Hip Innovation Technology, other from Alphaeon/Strathsby Crown, other from Elute, other from Ceribell, other from Acumed, other from PRN-Veterinary, other from Illuminus, other from Intellijoint, other from Osteal, other from Nanooxygenic, other from Sonata, other from Molecular Surface Technologies, other from Peptilogic, non-financial support from NIH, non-financial support from OREF, non-financial support from 3M, non-financial support from Aesculap, non-financial support from AO Spine, non-financial support from Biomet, non-financial support from Cempra, non-financial support from DePuy, non-financial support from Integra, non-financial support from Lima, non-financial support from Myoscience, non-financial support from NDRI, non-financial support from Novartis, non-financial support from Pfizer, non-financial support from Rotation Medical, non-financial support from Simplify Medical, non-financial support from Smith & Nephew, non-financial support from Stelkast, non-financial support from Stryker Orthopedics, non-financial support from Synthes, non-financial support from TissueGene, non-financial support from Tornier, non-financial support from Orthospace. All of these, outside the submitted work.

Other authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

D.F.R., S.T., and N.Z. prospectively collected the air swabs and the surgical-related variables. D.F.R, K.G., and J.P. wrote and editted the main manuscript text. D.F.R. prepared the figures and tables. All authors reviewed the manuscript.

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Figures

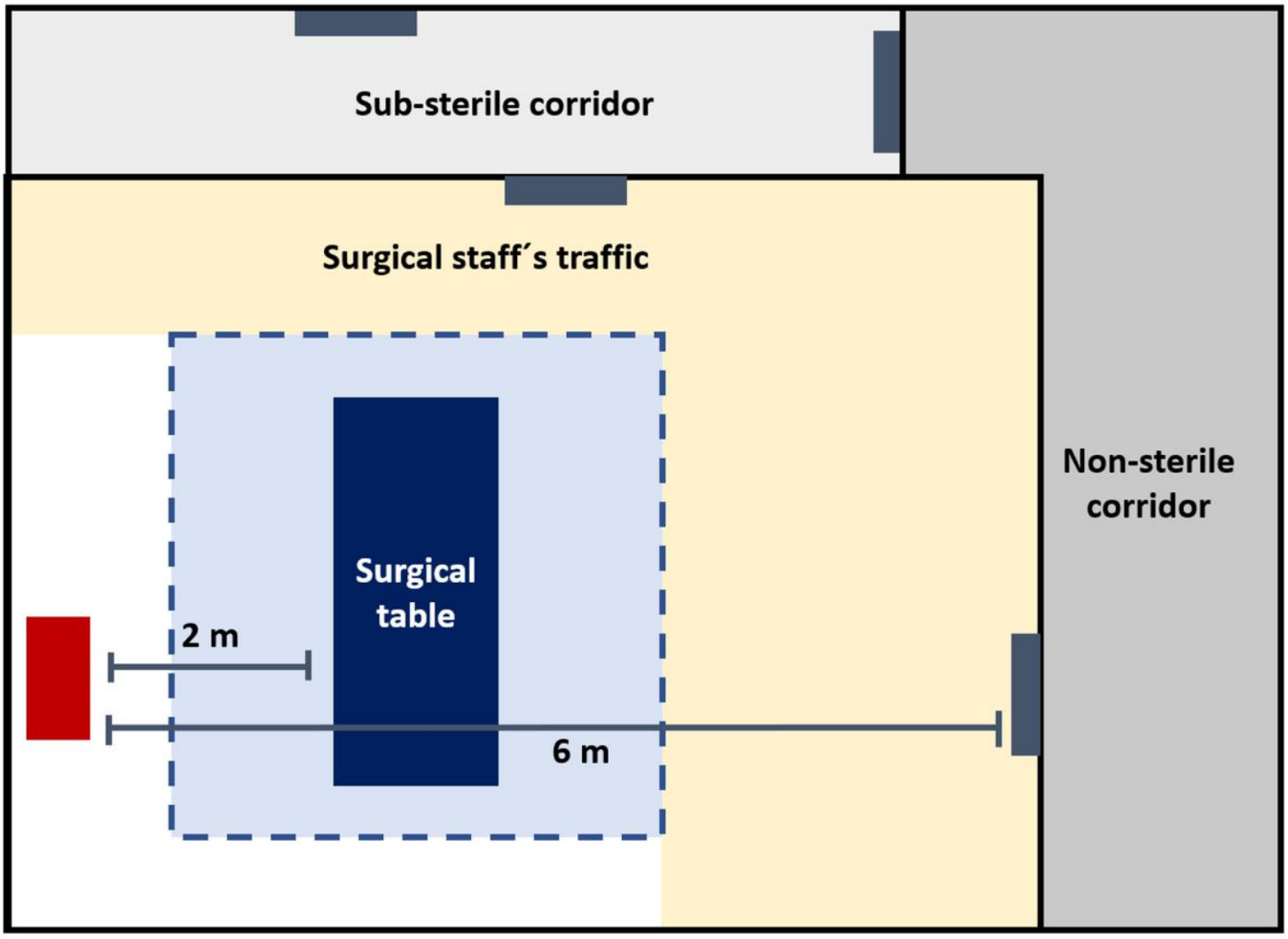

Figure 1

Operating room floor plan. The filtration unit with a crystalline ultraviolet (C-UVC) light is represented with a red rectangle and doors (source of contamination) are represented by dark gray rectangles.

Figure 2

Air flow and air swabs collection. Inflow air swabs were obtained from the bottom right part of the unit, while waving the swab at 45° from the floor. Outflow air swabs were collected from the upper central part of the unit, while waving the swab at 45° from the ceiling. All swabs were exposed for 3 minutes.