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ORIGINAL ARTICLE

A quantitative method to assess the role of indoor air decontamination to simultaneously reduce contamination of environmental surfaces: testing with vegetative and spore-forming bacteria

B. Zargar[1], S.A. Sattar[1,2], J.R. Rubino[3] and M.K. Ijaz[3,4]

1 CREM Co Labs, Units 1-2, 3403 American Drive, Mississauga, ON, Canada
2 Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada
3 RB, Montvale, NJ, USA
4 Department of Biology, Medgar Evers College of the City University of New York (CUNY), Brooklyn, NY, USA

Significance and Impact of the Study: The innovative and generic test protocol described can quantitatively assess the reduction in environmental surface contamination from microbial decontamination of indoor air in the same setting. This added advantage from air decontamination has implications for infection prevention and control in healthcare and other settings without the need for additional expense or effort. Continuous operation of an air decontamination device, such as the one tested here, can lead to ongoing reductions in pathogens in air and on environmental surfaces.

Keywords
airborne pathogens, indoor air, indoor air decontamination, preventing surface contamination, settling of airborne particles, surface contamination.

Correspondence
Bahram Zargar, Units 1-2, CREM Co Labs, 3403 American Dr, Mississauga, ON L4V 1T4, Canada.
E-mail: bzargar@cremco.ca

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Abstract

Indoor air can spread pathogens, which can be removed/inactivated by a variety of means in healthcare and other settings. We quantitatively assessed if air decontamination could also simultaneously reduce environmental surface contamination in the same setting. Two types of vegetative bacteria (*Staphylococcus aureus* and *Acinetobacter baumannii*), and a bacterial spore-former (*Geobacillus stearothermophilus*) were tested as representative airborne bacteria. They were separately aerosolized with a Collison nebulizer into a 24-m^3 aerobiology chamber and air samples collected with a programmable slit-to-agar sampler. Settling airborne particles were collected on culture plates placed at, and collected from, five different locations on the floor of the chamber with a custom-built remote plate-placement and -retriever system. Experimentally contaminated air in the chamber was decontaminated for 45 min with a device based on HEPA filtration and UV light. The plates were incubated and CFU counted. The device reduced the viability levels of all tested bacteria in the air by >3 $\log_{10}$ (>99·9%) in 45 min. Based on two separate tests, the average reductions in surface contamination for *S. aureus, A. baumannii* and *G. stearothermophilus* were respectively, 97, 87 and 97%. We thus showed that air decontamination could substantially and simultaneously reduce the levels of surface contamination in the same setting irrespective of the type of pathogen present.

Introduction

Indoor air is well recognized as a vehicle for the direct and indirect spread of a wide variety of human pathogens (Decraene *et al.* 2008; Sattar *et al.* 2016b; Zemouri *et al.* 2017), and many technologies are used to remove/inactivate such airborne pathogens in healthcare and other settings (Rautemaa *et al.* 2006). We had previously shown a HEPA filter- and UV-light-based indoor air decontamination device to reduce the levels of airborne vegetative

bacteria by ≥3·0 $\log_{10}$ (≥99·9%) in ~45 min (Ijaz *et al.* 2016; Sattar *et al.* 2016a; Zargar *et al.* 2016). In this study, the same device was tested to quantitatively assess if it could also reduce the microbial contamination of environmental surfaces in the same setting.

Results and discussion

During all tests, the air temperature was at 22 ± 2°C and the relative humidity (RH) at 55 ± 5%.

As shown in Table 1, the level of viable airborne bacteria achieved in the chamber air after 10 min of nebulization ranged between 4·4 $\log_{10}$ and 4·9 $\log_{10}$ CFU per m^3 of the air in the chamber. The air decontamination device was able to remove/inactivate all three bacteria tested by >3·0 $\log_{10}$ (>99·9%) in 45 min of its operation. Table 2 shows the levels of CFU on the control, first and second series of tested Petri plates held in the chamber. The air decontamination device could reduce the levels of surface contamination of *Staphylococcus aureus*, *Acinetobacter baumannii* and *Geobacillus stearothermophilus* respectively, by 97, 87 97% as compared to the controls.

This investigation has led to the development of a novel and quantitative protocol to assess the prevention of environmental surface contamination because of microbial decontamination of indoor air in the same setting. Its findings thus have important implications for infection prevention and control in healthcare and other settings. This would be especially relevant for mechanical indoor air decontamination devices operating on a continuous basis in a given setting such as a hospital ward, surgical suites, daycare centres, extended-care homes and consumer settings, thereby breaking the air-surface-air nexus. Therefore, the investigation revealed that targeting airborne pathogens could entail additional benefits, such as preventing or reducing the deposition of harmful micro-organisms on secondary vehicles that include frequently touched environmental surfaces and also preventing or potentially reducing their resuspension from these surfaces back into the air via a variety of indoor activities (Ijaz *et al.* 2016).

We believe that the technique reported is innovative in nature as it allows the placement and retrieval of Petri plates for airborne microbial sampling without breaking the containment barrier. Furthermore, it allows for a quantitative assessment of reduction/prevention of environmental surface contamination from airborne particles. It is also versatile enough for work with all major classes of airborne infectious agents. As far as we are aware, no such study is available in the published literature.

Table 1 Levels of airborne microbial contamination achieved in the aerobiology chamber after 10 min of nebulization

	$\log_{10}$ CFU per m^3 of chamber air		
Test bacteria	Control	Test 1	Test 2
Staphylococcus aureus	4·6	4·6	4·9
Acinetobacter baumannii	4·8	4·4	4·6
Geobacillus stearothermophilus	4·6	4·5	4·6

The chamber used was designed and built to conform to the guideline (2012) from the U.S. Environ. Protection Agency. While it may be somewhat smaller when compared to a standard surgical suite, it was large enough to represents many sites in healthcare and other settings. The use of larger aerobiology rooms can pose certain limitations. For example, higher concentrations of microbial pools are needed to achieve the desired level of contamination in the room air; this can be difficult and prohibitively expensive with certain types of airborne bacteria and viruses.

The vegetative bacteria tested represented two major classes of airborne pathogens. *Staphylococcus aureus* and *A. baumannii* are not only recognized in themselves as airborne pathogens (Eames *et al.* 2009; Yakupogullari *et al.* 2016), they also respectively, represented Gram-positive cocci and Gram-negative bacilli. The spores of *G. stearothermophilus*, an obligate thermophile (Sattar *et al.* 1972), acted as a surrogate for spore-forming airborne pathogens such as *Bacillus cereus*, and *Clostridium difficile* (Bottone 2010; Davies *et al.* 2011).

The culture plate placement and retrieval system described here made it possible to move the trays with the plates loaded in a smooth and simple fashion from outside the chamber and without breaking the containment barrier. All the hardware for the system was purchased locally and relatively inexpensively. The system could also be readily removed and reinstalled when required.

The study described has the potential for use with other types of airborne pathogens such as fungi, viruses and mycobacteria as well as work with bacteriophages as surrogates for viruses (Turgeon *et al.* 2014).

Decontamination of the air by the tested machine was based on a combination of mechanical removal by the HEPA filter and UV ray inactivation. While there are some difference between percent reductions of the three micro-organisms tested, the difference were not significant.

We regularly use the spores of *G. stearothermophilus* in our experiments and have not encountered any issues with their germination.

We chose the 2 h settling time based on our preliminary testing, which indicated that most of the airborne micro-organisms would settle down in that time period.

While this investigation focused on only one type of commercial device, its basic approach can be readily

Table 2 Levels of microbial contamination that settled on the Petri plates placed on the floor of the aerobiology chamber and percent reductions from air decontamination

	CFU on each plate*			CFU per m^2			Percent reduction	
Test bacteria	Control	Test 1	Test 2	Control	Test 1	Test 2	Test 1	Test 2
Staphylococcus aureus	159 ± 28	4·8 ± 3·5	8·8 ± 12	31840 ± 5681	994 ± 742	866 ± 1201	97·0 ± 2·0%	97·4 ± 3·5%
Acinetobacter baumannii	48 ± 11	2·8 ± 2·6	2·6 ± 1·8	2537 ± 2258	1442 ± 1314	937 ± 654	85·0 ± 10·0%	89·7 ± 2·5%
G. stearothermo-philus	97·8 ± 33	1·9 ± 1·7	3·5 ± 3·4	19553 ± 6496	351 ± 374·1	661 ± 640	97·4 ± 1·5%	98·0 ± 1·9%

*In each test, 30 Petri plates in five groups of six plates each were used. Therefore, the CFU per plate is the mean ± SD of 30 values for each test.

adapted for work with other existing or experimental technologies. However, when testing chemicals for indoor air decontamination, addition of a suitable neutralizer in the culture medium would be necessary to quench any microbial growth inhibition by chemical residues.

Materials and methods

The aerobiology chamber

The details of the chamber (Fig. 1) have been published before (Sattar *et al.* 2016a). Briefly, the chamber (24 m^3) was built to comply with the guidelines from the U.S. Environmental Protection Agency (U.S. EPA 2012). Between uses, fresh air was used to flush out the chamber of any residual airborne micro-organism.

A built-in pair of gloves and a transfer chamber on one side of the chamber permitted the handling of the required items without breaching the containment barrier. A muffin fan (Model A31022-20; 80 mm × 80 mm, with an output of 0·17 m^3 min^{-1}), purchased from Nidec Corp. (St. Louis, MO), was placed inside the chamber to enable the uniform mixing of the air inside it. The air temperature and RH inside the chamber were remotely measured and recorded using a remote-sensing device (Dickson Co., Addison, IL; http://www.dicksondata.com/products/wizard) allowed real-time monitoring and recording of these parameters; any changes could also be observed on a computer monitor placed in the vicinity of the larger aerobiology chamber.

The air decontamination device

The device (Germ Guardian AC4825; Guardian Technologies, Mentor, OH) was purchased in the open market and installed and used according to the manufacturer's instructions. It was based on a combination of HEPA filtration and UV irradiation. For this study, the device was placed in one corner of the aerobiology chamber and remotely operated at its highest speed (100 CFM).

The Collison nebulizer

A six-jet Collison nebulizer (CH Tech., Westwood, NJ; www.inhalation.org) was used to generate the aerosols of the test bacteria. The outlet of the nebulizer was connected via a PVC pipe into the center of the chamber and the nebulizer was operated for the desired length of time with air pressure (25 psi; 172·4 kPa) from a compressed air cylinder. The fluid to be nebulized consisted of a suspension of the test micro-organism in a soil load to simulate the presence of body fluids (ASTM 2011; Springthorpe and Sattar 2005).

The air sampler

A programmable surface-to-agar (STA) air sampler (Particle Measuring Systems, Boulder, CO; http://www.pmeasuring.com/home) was used to collect air samples from the aerobiology chamber at the rate of 28·3 l (1 ft^3) per min. The sampler was placed outside the chamber and the sampler's inlet was connected via a PVC pipe to withdraw air from the aerobiology chamber. A fresh plate (150 mm diameter) with a suitable nutrient agar was used to collect an air sample and the plates incubated for the development of CFU of the test bacteria. The air sample collection time varied from 2 to 10 min depending on the nature of the experiment.

Culture plate placement and retrieval system

A system was custom-designed to remotely place and retrieve culture plates in the four corners and center of the aerobiology chamber's floor. Using the transfer-box and gloves built into the chamber, each plastic tray (35 cm long × 25 cm wide and ×5 cm deep) with a clear plastic lid was loaded with six culture plates each. A 15 m-long wire (Steel Fish Tape with Illuminated end) was attached to one narrow side of the tray allowing for the movement of each tray on the metallic tracts (built using ProSTUD 20 1-1/4 in. × 12 ft. 20-gauge EQ

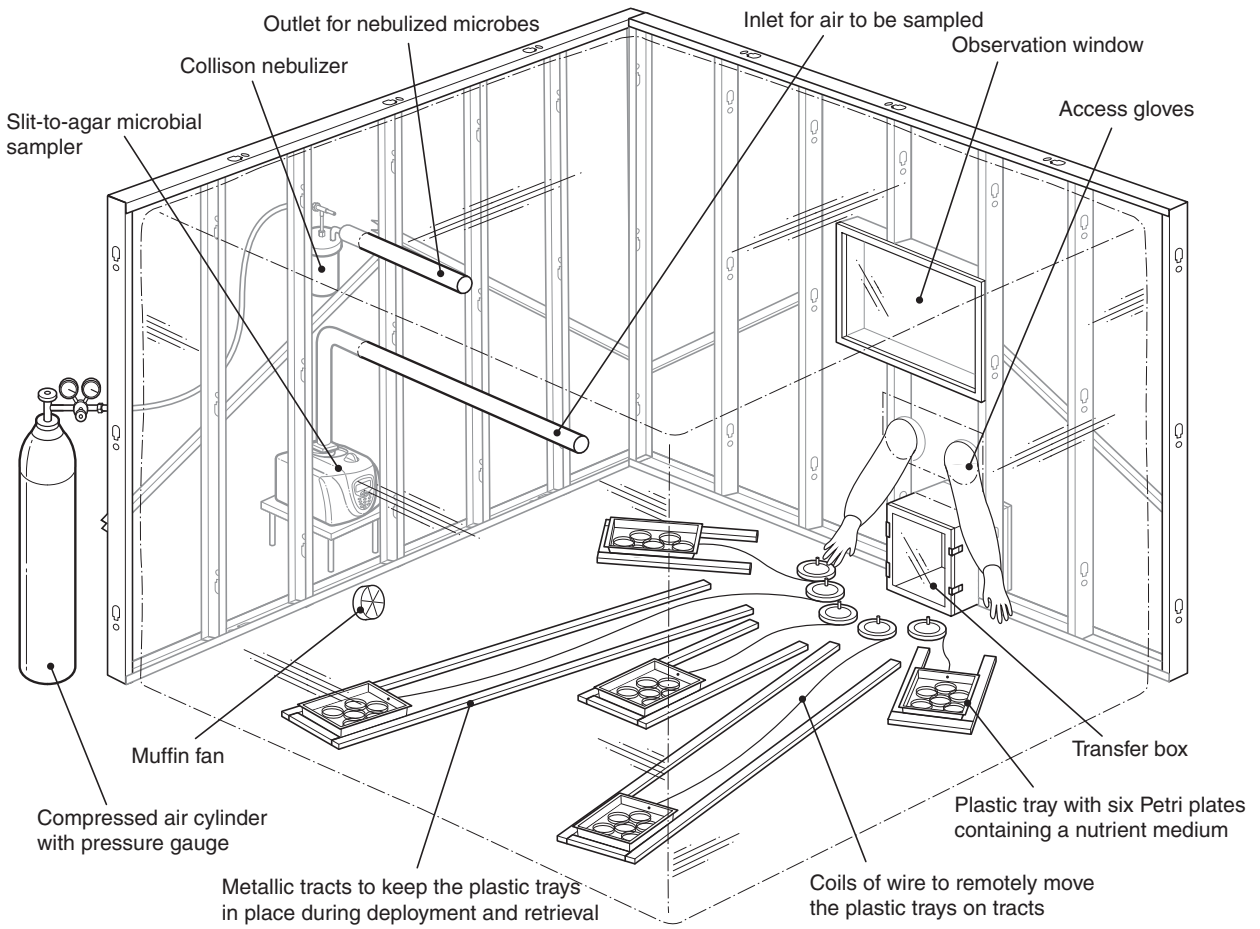


Figure 1 Aerobiology chamber with the entire Petri plate deployment set-up. Plastic trays with the Petri plates could be placed on metal tracts and remotely and individually moved with cables to five locations on the floor of the chamber. The lids on the plates were removed via the glove-box prior to their exposure to the air in the chamber, and replaced after the exposure was over. The device near the wall on the left is a muffin fan to circulate the air in the chamber.

galvanized steel wall framing stud). The tracts themselves were laid separately on the floor to allow the placement of the trays into the five locations (four corners and the center). Figure 1 depicts the layout of the aerobiology chamber with the culture plate placement and retrieval system installed; all the needed supplies for this system were purchased locally from a hardware supplier.

Culture of vegetative bacteria and the spore-former

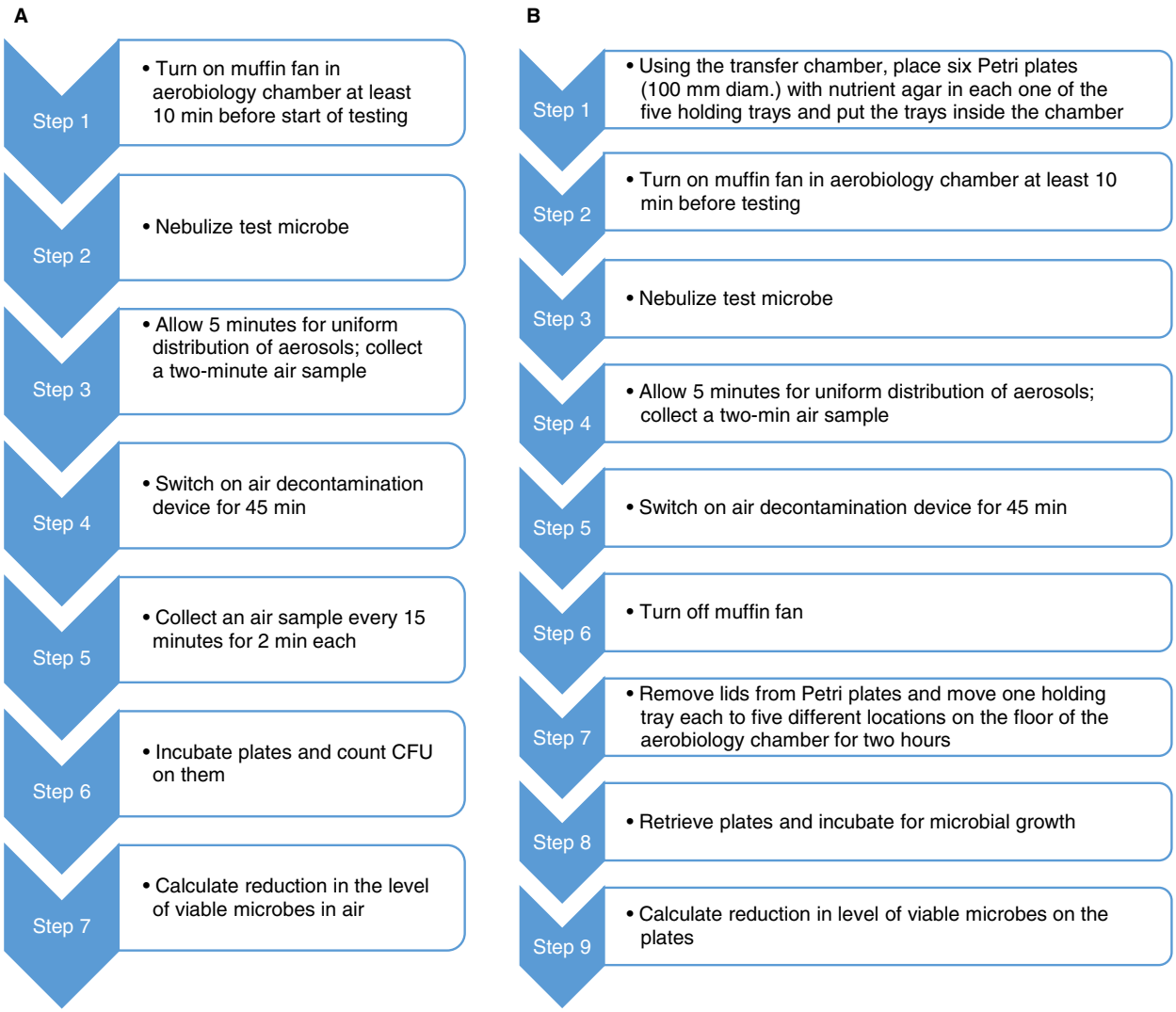
Staphylococcus aureus (ATCC 6538) and *A. baumannii* (ATCC #19606) were grown aerobically in trypticase soy broth (TSB; Oxoid) at 36 ± 1°C for 18 ± 2 h. The spores of *G. stearothermophilus* (ATCC 12980) were produced by growing it aerobically in TSB for 18 ± 2 h but at 56 ± 1°C; the broth culture was heated at 60 ± 1°C for 30 min in a waterbath to inactivate any vegetative cells.

Experimental setup

Flow chart 1 provides the sequence of steps in a typical experiment. Each test for air decontamination and reduction in surface contamination was performed twice with each one of the three types of bacteria tested. In each test to assess surface contamination, 30 culture plates were used. Therefore, the CFU counts reported represent a mean ± SD of 30 individual values.

Determination of the background levels of contamination of Petri plates from nebulized bacteria

As shown in Flow chart A, the test microbial suspensions were separately nebulized into the chamber with the muffin fan operating for 10 min to evenly distribute the airborne contamination. A 2-min air sample was collected from the chamber using an STA sampler to determine the



initial level of bacteria in it. Another 45 min were allowed for circulation of the airborne micro-organisms in the chamber (the time required for the device to achieve a 3 $\log_{10}$) reduction in CFU in the air. At the end of this time, thirty TSA plates, in groups of six each, were placed on the floor of the aerobiology chamber, with one set in each of the four corners and one in the center. The lids of the plates were removed. The muffin fan was then turned off and 120 min allowed for the airborne micro-organisms to settle. At the end of this period, the Petri plates were retrieved, their lids replaced for incubation at the required temperature for CFU development to determine the level of microbial contamination deposited on each one. Such testing allowed us to determine the levels of airborne bacteria that could settle on the plates without any air decontamination. One plate of unexposed TSA was incubated for 24 h at 36 ± 1°C to check for sterility of the culture medium.

Assessing the activity of the tested device in preventing surface contamination from airborne bacteria

As summarized in Flow chart B, the challenge bacterium was first suspended in the nebulizing fluid. It was then nebulized into the chamber with the muffin fan operating for 10 min to evenly distribute the aerosolized test microbial agent. A 2-min air sample was collected from the chamber using an STA sampler to determine the level of airborne contamination. The test air decontamination device was turned on in the chamber and allowed to work for 45 min. A total of 30 plastic Petri plates (100 mm diam. each), in groups of six each, were placed on the floor of the chamber as mentioned above. The lids of the plates were removed. The device and the muffin fan were turned off and 120 min allowed as sufficient time for any airborne bacteria to settle. At the end of this period, the Petri plates were retrieved and their lids replaced for incubation at the required

incubation temperature for CFU development to determine the level of microbial contamination deposited on each plate. A plate of non-exposed TSA was incubated for 46 ± 2 h to check the sterility of the agar medium.

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

Bottone, E.J. (2010) *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev* **23**, 382–398.

Davies, A., Pottage, T., Bennett, A. and Walker, J. (2011) Gaseous and air decontamination technologies for *Clostridium difficile* in the healthcare environment. *J Hosp Infect* **77**, 199–203.

Decraene, V., Ready, D., Pratten, J. and Wilson, M. (2008) Air-borne microbial contamination of surfaces in a UK dental clinic. *J Gen Appl Microbiol* **54**, 195–203.

Eames, I., Tang, J.W., Li, Y. and Wilson, P. (2009) Airborne transmission of disease in hospitals. *J R Soc Interface* **6** (Suppl 6), S697–S702.

Ijaz, M.K., Zargar, B., Wright, K.E., Rubino, J.R. and Sattar, S.A. (2016) Generic aspects of the airborne spread of human pathogens indoors and emerging air decontamination technologies. *Am J Infect Control* **44**(9 Suppl), S109–S120.

Rautemaa, R., Nordberg, A., Wuolijoki-Saaristo, K. and Meurman, J.H. (2006) Bacterial aerosols in dental practice - a potential hospital infection problem? *J Hosp Infect* **64**, 76–81.

Sattar, S.A., Synek, E.J., Westwood, J.C. and Neals, P. (1972) Hazard inherent in microbial tracers: reduction of risk by the use of *Bacillus stearothermophilus* spores in aerobiology. *Appl Microbiol* **23**, 1053–1059.

Sattar, S.A., Ijaz, M.K. and Bhardwaj, N. (2016b) Airborne viruses. In *Manual of Environmental Microbiology*, 4th edn. pp. 3.2.7-1–3.2.7-24. Washington, DC: Am. Soc. Microbiol.

Sattar, S.A., Kibbee, R.J., Zargar, B., Wright, K.E., Rubino, J.R. and Ijaz, M.K. (2016a) Decontamination of indoor air to reduce the risk of airborne infections: studies on survival and inactivation of airborne pathogens using an aerobiology chamber. *Am J Infect Control* **44**, e177–e182.

Springthorpe, V.S. and Sattar, S.A. (2005) Carrier tests to assess microbicidal activities of chemical disinfectants for use on medical devices and environmental surfaces. *J AOAC Int* **88**, 182–201.

Turgeon, N., Toulouse, M.J., Martel, B., Moineau, S. and Duchaine, C. (2014) Comparison of five bacteriophages as models for viral aerosol studies. *Appl Environ Microbiol* **80**, 4242–4250.

U.S. Environmental Protection Agency (2012) *Air Sanitizers – Efficacy Data Recommendations*. OCSPP 810.2500. Washington, DC: U.S. EPA.

Yakupogullari, Y., Otlu, B., Ersoy, Y., Kuzucu, C., Bayindir, Y., Kayabas, U., Togal, T. and Kizilkaya, C. (2016) Is airborne transmission of *Acinetobacter baumannii* possible: a prospective molecular epidemiologic study in a tertiary care hospital. *Am J Infect Control* **44**, 1595–1599.

Zargar, B., Kashkooli, F.M., Soltani, M., Wright, K.E., Ijaz, M.K. and Sattar, S.A. (2016) Mathematical modeling and simulation of bacterial distribution in an aerobiology chamber using computational fluid dynamics. *Am J Infect Control* **44**(9 Suppl), S127–S137.

Zemouri, C., de Soet, H., Crielaard, W. and Laheij, A. (2017) A scoping review on bio-aerosols in healthcare and the dental environment. *PLoS ONE* **12**, e0178007.