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Major Article

Decontamination of indoor air to reduce the risk of airborne infections: Studies on survival and inactivation of airborne pathogens using an aerobiology chamber

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Background: Although indoor air can spread many pathogens, information on the airborne survival and inactivation of such pathogens remains sparse.
Methods: *Staphylococcus aureus* and *Klebsiella pneumoniae* were nebulized separately into an aerobiology chamber (24.0 m^3). The chamber's relative humidity and air temperature were at 50% ± 5% and 20°C ± 2°C, respectively. The air was sampled with a slit-to-agar sampler. Between tests, filtered air purged the chamber of any residual airborne microbes.
Results: The challenge in the air varied between 4.2 $\log_{10}$ colony forming units (CFU)/m^3 and 5.0 $\log_{10}$ CFU/m^3, sufficient to show a ≥3 $\log_{10}$ (≥99.9%) reduction in microbial viability in air over a given contact time by the technologies tested. The rates of biologic decay of *S aureus* and *K pneumoniae* were 0.0064 ± 0.00015 and 0.0244 ± 0.009 $\log_{10}$ CFU/m^3/min, respectively. Three commercial devices, with ultraviolet light and HEPA (high-efficiency particulate air) filtration, met the product efficacy criterion in 45-210 minutes; these rates were statistically significant compared with the corresponding rates of biologic decay of the bacteria. One device was also tested with repeated challenges with aerosolized *S aureus* to simulate ongoing fluctuations in indoor air quality; it could reduce each such recontamination to an undetectable level in approximately 40 minutes.
Conclusions: The setup described is suitable for work with all major classes of pathogens and also complies with the U.S. Environmental Protection Agency's guidelines (2012) for testing air decontamination technologies.

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Air, a universal environmental equalizer, affects all living and nonliving forms. For humans, it has profound health implications in all indoor environments, where we spend most of our time.[1-3] Indoor air quality is also forever changing because of the influence of many controllable and uncontrollable factors, which are virtually everywhere. Indoor air, in particular, can expose us to noxious chemicals, particulates, pollen, allergens, and a variety of infectious agents.[4,5] Emerging pathogens, such as *Acinetobacter baumannii*,[6,7] noroviruses,[8] and *Clostridium difficile*,[9] have also been detected in indoor air, with a strong potential for airborne dissemination. Therefore, there is renewed emphasis on the potential of indoor air for transmitting many types of infectious agents by direct inhalation.[10,11] Also, airborne pathogens may settle on environmental surfaces, which could then become secondary vehicles indoors.[7,12] The possible transmission of drug-resistant bacteria by indoor air adds another cause for concern.[7,13] A combination of ongoing societal changes is also enhancing the potential of air as a vehicle for pathogens.[14,15] Therefore, indoor air can play a significant role in the direct and indirect transmission of a variety of human pathogens in health care and in other institutional and domestic settings.

This widening recognition of indoor air as a potential vehicle for pathogens is leading to a corresponding upsurge in the marketing of products and technologies with claims for safe and effective air decontamination.[2] However, scientifically valid and standardized protocols remain unavailable to generate field-relevant data for label

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claims and for their review for infection preventionists and consumer and regulatory purposes. This study was initiated to address the gap.

MATERIALS

Any item requiring steam sterilization prior to use was autoclaved at 121°C for 45 minutes. All disposable labware that was contaminated with infectious materials was autoclaved prior to disposal as biomedical waste.

A soil load[16] was added to all microbial suspensions that were nebulized into the chamber. Separate stock solutions of bovine serum albumin (BSA; Sigma-Aldrich, St Louis, MO), mucin from bovine submaxillary glands (Sigma-Aldrich), and yeast extract powder type 1 were prepared by dissolving 0.5, 0.04, and 0.5 g, respectively, in 10 mL of Dulbecco phosphate-buffered saline (DPBS; pH, 7.2 ± 0.2). The solutions were individually passed through a syringe-mounted polyethersulfone (Sterlitech, Kent, WA) membrane (0.2 µm in pore diameter), aliquoted as 1.5-mL volumes, and stored at –20 ± 2°C with a shelf life of at least 1 year.

Table 1
Specification on the air decontamination devices tested

Device no.	Flow rate, m^3/min (ft^3/min)	Time to expose entire contents of the chamber once	Theoretical no. of exposures in 8 h of an aerosol particle	Ultraviolet light bulb wattage
1	2.8 (100)	0.14 h (8.6 min)	55.9	5 (LB 4000)
2	3.4 (12)	0.12 h (7.2 min)	66.7	8 (LB 5000)
3	1.7 (60)	0.24 h (14.3 min)	33.5	9 (ZW6S12W)

Staphylococcus aureus (ATCC 6538; ATCC) and *Klebsiella pneumoniae* (ATCC 4352; ATCC) were used as representative airborne pathogens as recommended by the U.S. Environmental Protection Agency (EPA).[17] These microbes represent common types of airborne gram-positive and gram-negative pathogens, respectively.

Trypticase soy broth (TSB; Oxoid, Basingstoke Hampshire, UK) was used to culture both of the microorganisms, and Modified Letheen Agar (Thermo Fisher Scientific, Waltham, VA) in 150-mm disposable plastic Petri plates (Thermo Fisher Scientific) was used for their recovery from the air. The same agar medium in 100-mm disposable plastic Petri plates (Thermo Fisher Scientific) was used to assay the bacterial suspensions for their colony forming units (CFUs). All cultures were incubated at 36°C ± 1°C, and observed after 18 ± 2 hours of incubation and then again at the end of 5 days to detect the presence of any late-growing bacterial cells.

Testing of air decontamination technologies

We assessed 3 types of commercial devices marketed for air decontamination. Table 1 summarizes the basic features of the tested devices. These devices were purchased in the open market and operated according to the manufacturer's instructions. Each test device was placed inside the aerobiology chamber and operated remotely.

AEROBIOLOGY CHAMBER

The aerobiology chamber (Fig 1), with a volume of 24.3 m^3 (860.0 ft^3), was located inside a biosafety containment level 3 facility. The materials to build it were all purchased locally. One layer of polyethylene sheeting (0.1524-mm [0.006-in] thick) was affixed

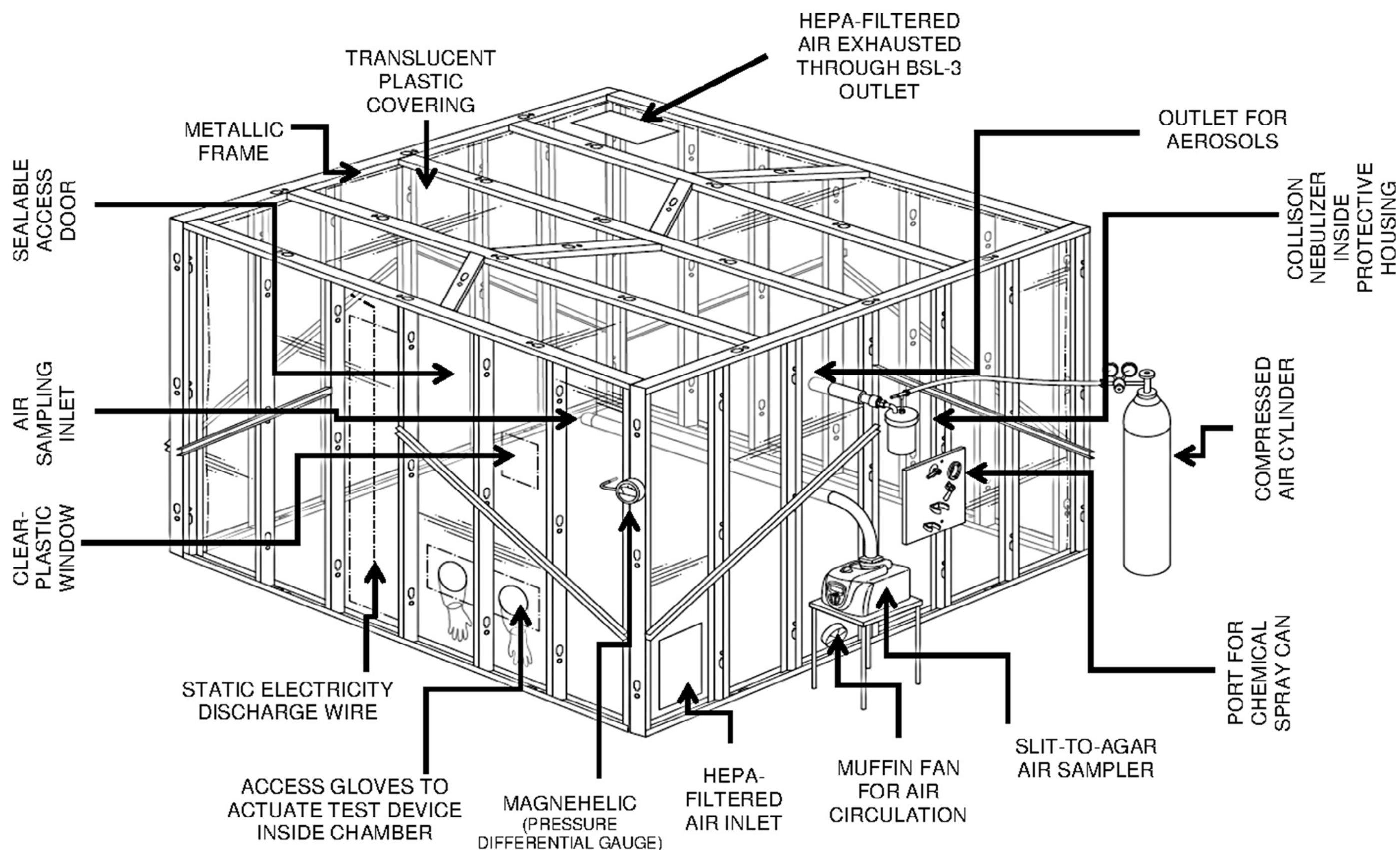

Fig 1. Aerobiology chamber with essential components (length × width × height, 320.0 × 360.6 × 211.0 cm; 24.3 m^3 [860 ft^3]). *BSL-3*, biosafety containment level 3. *HEPA*, high-efficiency particulate air.

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to the inside of a steel-framed structure with polyvinyl chloride adhesive tape and plastic ties, with another layer over it to represent the walls, ceiling, and floor to maintain an airtight seal. Sealable windows or doors provided access to the inside of the chamber for maintenance and for placement and removal of any monitoring and test devices that were used. The plastic sheeting could be easily and safely removed, decontaminated as biohazardous waste by autoclaving, and discarded when no longer required.

The sheeting was grounded with a copper wire to dissipate any static electricity. Similarly, the copper wire that was used to suspend the polyvinyl chloride air sampling pipe acted as a grounding wire. A muffin fan, placed on the floor of the chamber directly underneath the nebulizer inlet pipe, was actuated from the outside for operation during nebulization and throughout a given test for uniform distribution of the aerosolized particles into the air inside. The chamber's internal environment was monitored throughout an experiment with a wireless relative humidity, air temperature sensor, and data logger system (Dickson, Addison, IL) and recorded on a computer for subsequent download and analysis.

Between experiments, the chamber was purged with HEPA-filtered (high-efficiency particulate air) air for a minimum of 1 hour to remove any microbe-laden airborne particles, and the exhaust passed through a HEPA-filtered ventilation system.

METHODS

To prepare frozen stocks of the test bacteria, tubes with 9 mL of TSB were separately inoculated and incubated at 36°C ± 1°C for 18 ± 2 hours. The resulting cultures were mixed with 1.0 mL of sterilized glycerol to yield a 10% (vol/vol) concentration of the cryopreservative, aliquoted into cryovials in 0.5 mL volumes for storage at –80°C ± 2°C. For working stocks, a vial of frozen stock was thawed, 100 μL of it was placed into 10 mL of TSB, and the tube was incubated for 18 ± 2 hours. This represented the refrigerated stock for storage at 4°C ± 2°C and was used over a period of 6 ± 1 days.

To prepare a bacterial suspension for nebulization, 10 mL of TSB received 100 μL of the refrigerated stock and was always incubated for 20 hours for a consistent yield in CFU. For *S aureus*, 50 μL of the culture was then added to 10.14 mL of DPBS along with 0.75 mL BSA, 1.05 mL yeast extract, 3.0 mL mucin, and 10 μL of Antifoam A Concentrate (Sigma-Aldrich) for a total of 15.0 mL. Because *K pneumoniae* was less stable than *S aureus* during nebulization and also once aerosolized, 150 μL of the 20-hour culture was added to 10.04 mL of DPBS to achieve a sufficiently high input titer of CFU in the chamber while keeping the volume of the other components the same. To quantify the level of viable bacteria aerosolized into the chamber, the fluid in the nebulizer was titrated for CFU after spraying using the Miles and Misra method.[18]

Aerosolization of test bacteria

A 6-jet Collison nebulizer (CH Technologies, Westwood, NJ), which generates particles in the respiratory range (0.5-5.0 μm), was used to spray the test bacteria separately into the chamber.

Sampling of air for viable microbes

To assess the airborne survival of the test microbes or to determine the activity of the air decontamination devices, the air in the chamber was collected at the rate of 28.3 L/min (1 ft^3/min) using an externally placed slit-to-agar (STA) sampler (Particle Measuring Systems, Boulder, CO) for a time-related profile of viable bacterial content in the chamber's air. The collection times of each sample and the total number of samples collected depended on the type of test being performed.

Testing microbial survival in air

Testing was carried out first to determine the rate of biologic decay of the test microorganism(s) under the experimental conditions to be used for testing potential air decontamination technologies. For this, the test microbe was aerosolized into the chamber, and a series of air samples were collected using an STA sampler.

Data analyses

To compare the result of the efficacy tests with the stability-in-air test for each microorganism, a 1-way analysis of covariance was carried out on the data using the aoctool function in the MATLAB statistics toolbox (MathWorks, Natick, MA).

EXPERIMENTAL AND RESULTS

Testing microbial survival

The test microbial suspension in the soil load was aerosolized into the chamber; 2-minute air samples were collected and CFU on the recovery plates were counted after an incubation of 18 ± 2 hours. The information from 3 separate tests with both types of microorganisms is summarized in Figure 2. The rates of biologic decay for *S aureus* and *K pneumoniae* were found to be 0.0064 ± 0.00015 and 0.0244 ± 0.009 $\log_{10}$ CFU/m^3/min, respectively. No CFU of *K pneumoniae* could be detected after 210 minutes, whereas *S aureus* remained viable even after 480 minutes. Therefore, the rate of biologic decay of *K pneumoniae* was statistically significantly faster ($P = .0055$) than that of *S aureus*.

Testing microbial inactivation by the devices

Two separate units of device 1 and device 2 were tested. Because they behaved in the same manner, the data are presented as averages in Figures 3A (*S aureus*) and 3B (*K pneumoniae*).

As seen in Figure 3A, the average input titer of *S aureus* into the chamber was 4.61 CFU/m^3, and devices 1 and 2 could achieve a ≥ 3 $\log_{10}$ reduction in the viability of this microbe in 45 minutes, with no recoverable CFU from the air in the chamber after 60 minutes. However, device 3 produced a ≥ 3 $\log_{10}$ reduction in viability in 215 minutes, with CFU recoverable from the chamber's air even after 180 minutes.

The results of the experiments with *K pneumoniae* are shown in Figure 3B. The average input titer was 4.62 CFU/m^3, and device 1 could reduce the CFU level of the test microbe by ≥3 $\log_{10}$ in 45 minutes, with no CFU detectable after 60 minutes. The performance of device 2 (Fig 3B) against both of the challenge microbes was very similar to that of device 1. Device 3 produced a 3 $\log_{10}$ reduction in viability in 200 minutes, with CFU recoverable from the chamber's air even after 120 minutes.

Testing with repeated microbial challenge

In this experiment, device 1 was tested for its ability to deal with ongoing fluctuations in the microbiologic quality of indoor air. A suspension of *S aureus* was nebulized into the chamber at 3 separate time points while the device was left to operate continuously. The device efficacy after the 3 challenges was almost the same. The time at which the device demonstrated 3 $\log_{10}$ reductions after each nebulization was also calculated and found to be approximately 40 minutes. The mean of the 3 $\log_{10}$ reduction times was 40.13 ± 0.71, giving an average biologic decay rate of aerosolized bacteria after the 3 nebulizations of 0.0753 ± 0.0024 CFU/m^3/min.

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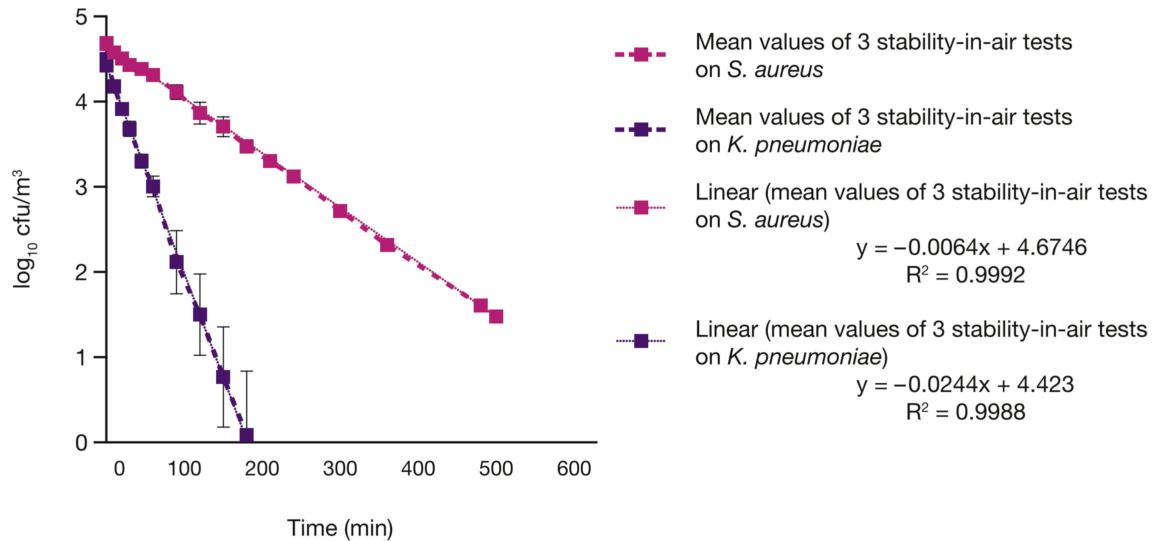

Fig 2. Biologic decay of *Staphylococcus aureus* and *Klebsiella pneumoniae* in the air of the chamber. *cfu*, colony forming units.

DISCUSSION

The study of microbial survival in indoor air and the proper assessment of methods for its decontamination present numerous challenges along with the need for specialized equipment, technical skills, and test protocols. Consequently, proper expertise and suitable experimental facilities for such investigations remain uncommon.

Here, we describe the setup and use of a relatively simple aerobiology chamber to study microbial survival in indoor air and also to test technologies that make claims for indoor air decontamination. Although the setup described has been successfully used with a variety of experimental and commercial indoor air decontamination technologies, and other types of microbes, the details presented here relate to work with 2 types of vegetative bacteria as recommended in the U.S. EPA's 2012 product performance guideline.[17] This guideline specifies the size of a sealed enclosure for experimental contamination of the air with microbial aerosols of vegetative bacteria to assess technologies for a temporary reduction in the load of airborne microbes. *S aureus* (ATCC 6538) and *K pneumoniae* (ATCC 4352) are recommended to assess indoor air decontamination technologies.

The Collison nebulizer is well-recognized as a standard device for the generation of microbial aerosols in the respirable range (0.1-5.0 µm in diameter). The liquid to be aerosolized consisted of the test microbial suspension and a soil load together with antifoam to minimize frothing during nebulization.

Pathogens become airborne either by direct ejection from infected or colonized individuals or by resuspension of already dried body fluids in the environment. Therefore, pathogens in air are almost always embedded in droplet nuclei, along with varying levels and types of organic and inorganic materials—the soil load. Therefore, any air decontamination technologies being assessed must be potent enough to reduce the pathogen load to the desired level while also coping with the soil load. The soil load used here was a mixture of BSA, bovine mucin, and yeast extract in a buffered solution. Albumin is a large molecular weight (66.5 kDa) protein that is common in body fluids. Mucin, a mucilaginous substance, is also common in body fluids and often protects microbes in body fluids against deleterious physical and chemical factors. The yeast extract represents peptides that result from the breakdown of proteins, and the amino acid residues in them can react with and neutralize the microbicidal activities of chemicals. The total protein content of the soil load is equal to that in 5% fetal calf serum that is often used as soil load. This soil load is already a part of 5 standards of the ASTM International[16] and a guide that was recently issued by the Organization for Economic Cooperation Development.[19]

Although there are several devices available for collecting and sizing particles that contain viable bacteria in air,[20] use of the programmable STA sampler provided the following advantages over the other devices: (1) direct capture of airborne particles on the surface of a suitable recovery agar medium containing ≥1 chemicals for immediate neutralization of any active(s), (2) air sample collection time could be programmed to last from a minimum of 2 minutes to a maximum of 5 hours, (3) in-line HEPA filter to trap any viable bacteria in the sampler's exhaust, (4) built-in vacuum pump to provide a stable air sampling rate of 28.3 L/min (1 ft^3/min), (5) ready retrieval and placement of Petri plates (Fisher) during a sampling session, (6) event-related profile of viable bacterial content in the air being sampled, (7) direct incubation of recovery plates for the development and counting of CFU, (8) wipe down with a disinfectant-soaked towelette was sufficient for decontamination between sample collections, and (9) automatic adjustment of the optimal distance between the slit and the surface of the agar for efficient particle capture throughout the sampling time.

Because this study focused solely on the testing of devices that did not introduce any chemicals into the air of the chamber, no validation of any chemical actives was needed in the aerosol recovery medium. However, the basic design of the chamber makes it feasible to test technologies based on microbicidal chemicals as well. For example, a fogger or spray device can be fixed to a built-in port (Fig 1) and trigger-activated to release the formulation. Alternatively, a fogger or spray device could be placed in the chamber and operated with the help of arm-length gloves installed on one side of the chamber.

The use of a remote-sensing relative humidity and air temperature meter placed inside the chamber allowed real-time monitoring and recording of the data; any ongoing changes could also be observed on a computer monitor placed outside.

As shown in Table 1, the 3 tested devices differed in their ultraviolet light output and the rate with which they could recycle the room air. Although device 3 had the highest ultraviolet light bulb wattage but the slowest fan speed, it took longer to achieve the product performance criterion (3.0-$\log_{10}$/99.9% reduction) than the

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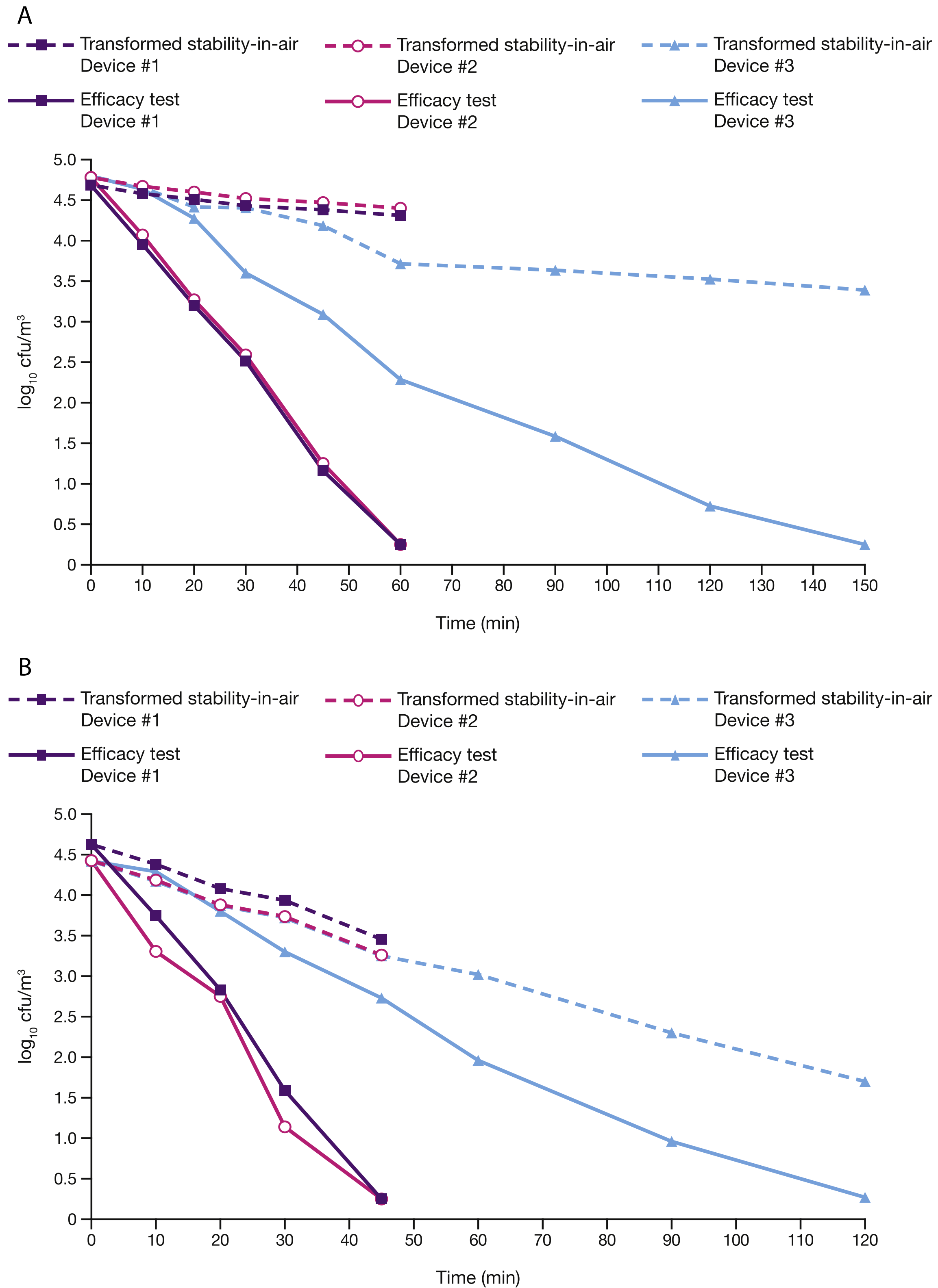

Fig 3. (A) Inactivation of airborne *Staphylococcus aureus* using 3 devices. (B) Inactivation of airborne *Klebsiella pneumoniae* using 3 devices. *cfu*, colony forming units.

other 2 devices. To confirm the role of the rate of air circulation, further testing will be needed at slower fan speeds.

The use of the programmable STA sampler with the minimum possible air sampling time of 2 minutes limits the initial number of CFU that can be used to contaminate the chamber. Nevertheless, the level of microbial challenge used in this study was high enough to show the ≥3.0 $\log_{10}$ (99.9%) reduction in viability, as recommended in the U.S. EPA's product performance guideline in

relation to an untreated control.[17] This level of microbial challenge was also selected based on a summary of the findings from 23 studies on indoor air in hospitals, which showed that the mean levels of viable bacteria range from 7-1,224 CFU/m^3.[2] The World Health Organization regards >50 and >100 CFU/m^3 of fungi and bacteria, respectively, in hospital air as potentially unsafe for human health.[2]

The recovery plates were incubated for 18 ± 2 hours, which was long enough to allow for development of countable colonies while reducing the risk of overgrowth. The plates with few or no colonies were incubated for a total of 5 days to allow any slow-growing stressed or injured bacteria to form colonies.

The 3 species of bacteria (*S aureus*, *K pneumoniae*, and *Pseudomonas aeruginosa*), as recommended for testing by the U.S. EPA,[17] are recognized airborne pathogens. However, *A baumannii* should also be considered in any future studies on decontamination of indoor air because of its mounting significance as a health care–associated pathogen.[6,7]

CONCLUSIONS

The study described conforms to the requirements as specified by the U.S. EPA in its guidance document.[17] The experimental setup used is also versatile enough to allow for testing against other types of microorganisms, including viruses, fungi, and spore-forming microorganisms, in addition to the ones recommended in the U.S. EPA's product performance guideline.[17]

Acknowledgments

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