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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.

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

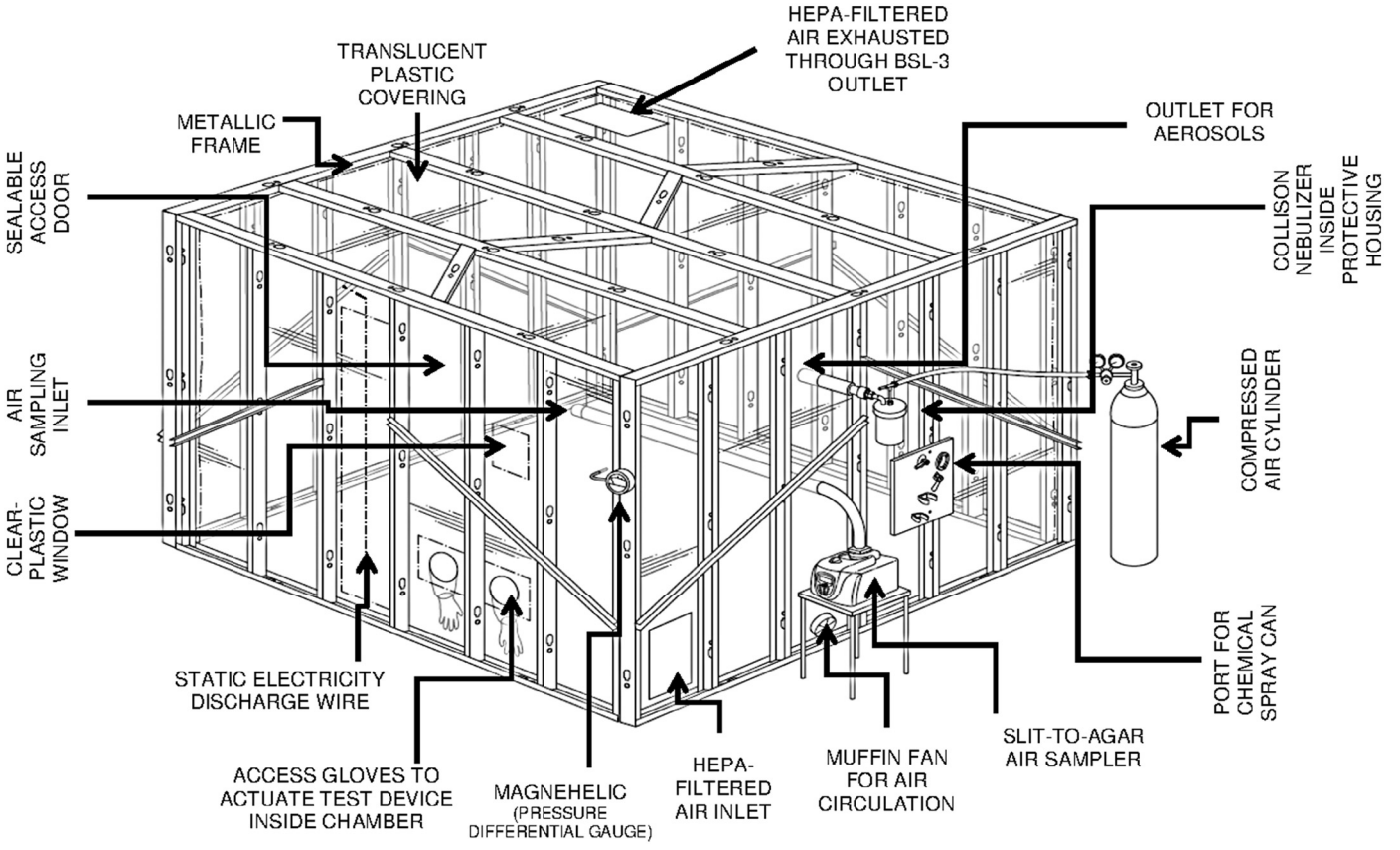

Fig 1. Aerobiology chamber with essential components (length × width × height, 320.0 × 360.6 × 211.0 cm; 24.3 m^3 [860 ft^3]). *BSL-3*, biosafety containment level 3. *HEPA*, high-efficiency particulate air.