Survival of Microorganisms on HEPA Filters

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Abstract

High Efficiency Particulate Air (HEPA) filters are required to minimize the release of microorganisms from laboratories and other settings. This study was carried out to assess whether a range of microorganisms captured on HEPA filters would survive under normal operating conditions. Bacillus atrophaeus *(NCTC 10073),* Staphylococcus epidermidis *(NCIMB 12721), MS-2 coliphage,* Escherichia coli *(NCIMB 9481),* Brevundimonas diminuta *(NCIMB 11091), and* Aspergillus brasiliensis *(ATCC 16404) were individually aerosolized using a Collison nebulizer and captured on HEPA filter material. Clean air was drawn through the loaded filters for 6 days at a constant rate (face velocity of 0.4-0.5 m/s^{-1}) for all organisms and for 210 days for* B. atrophaeus *to simulate the use of a HEPA filter. Pre-packed sterile filters were also contaminated with* B. atrophaeus *which survived on the HEPA filter material for 210 days with no significant loss of viability. MS-2 coliphage and* A. brasiliensis *survived over the 6 days with no significant loss of viability. There was a 5-log reduction in viability of* S. epidermidis *over 6 days, while both Gram-negative bacteria,* E. coli *and* B. diminuta*, were not recoverable after 48 hours of exposure. This study highlights the need for risk assessments and rigorous guidelines on the use and handling of air filtration membranes exposed to resistant pathogenic agents to minimize the risks of occupational exposure.*

Keywords

HEPA filters, air microbiology, aerosols, biosafety, filter material

Introduction

The use of HEPA filters in microbiological laboratory heating, ventilation, and air-conditioning (HVAC) systems is critical for the containment of airborne pathogens. The major role of HEPA filters in laboratories is to remove a range of microorganisms from the air and to prevent their release into uncontrolled areas (Abraham et al., 1998; Maus et al., 2001). The integrity and efficiency of HEPA filters are evaluated at regular intervals to determine that their performance is satisfactory. These evaluations involve an annual assessment of the integrity of the filter medium to determine that the filter is still retained in its housing and that the pressure drop across the filter is acceptable. When the filters are handled for assessment or removal, there is potential for exposure through re-aerosolization of microorganisms that may have been deposited onto the HEPA filter membrane surface during usage (Bearg, 1993; Neumeister et al., 1997).

This study set out to assess the survival of a range of microorganisms including bacteria, bacteriophage, and moulds on filter membranes. New HEPA filter materials were challenged with microbial aerosols under defined conditions and placed on a modular air-flow system to simulate use over a range of time periods.

A range of microorganisms (*Bacillus atrophaeus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Brevundimonas diminuta*, *Aspergillus brasiliensis*, and MS-2 coliphage) were selected to represent spore formers, vegetative bacteria, moulds, and viruses that could present a challenge to filters (Fannin et al., 1985; Laitinen et al., 1994; Maus et al., 2001) currently used in HVAC systems.

Materials and Methods

Stock cultures of *S. epidermidis* (NCIMB 12721) and *E. coli* (NCIMB 9481) (stored at 4ºC) were grown in Trypticase Soy Broth (TSB) (100 ml) in a shaking water bath (130 rpm, 37ºC for 19 hours ± 1 hour) to a concentration of 1.17 x 10^{10} cfu/ml^{-1} (nomenclature for "cfu per ml") and 3.15 x 10^{9} cfu/ml^{-1}, respectively.

Coliphage MS-2 (NCIMB 10108) was replicated using *E. coli* (NCIMB 9481) as the host. *E. coli* was subcultured from TSBA agar (37º ± 2ºC for 20 hours) into TSB (60 ml) and placed into a shaking incubator (120 rpm, 150 minutes at 37º ± 2ºC). Coliphage was then prepared by inoculating MS-2 stock into TSB which was aerated by shaking (37º ± 2ºC for 3 hours). The suspension was then centrifuged twice at 2,000 g (20 minutes) to remove the cell debris. The supernatant was transferred to a fresh flask and the concentration of phage was then determined.

An aqueous spore suspension (3.53 x 10^{9} spores/ml^{-1}) of *B. atrophaeus* (NCTC 10073), previously produced as a stock solution by Health Protection Agency (HPA) (Porton Production Division, Salisbury, UK) was used as the stock spore suspension for this study. Serial dilutions were prepared in phosphate buffer containing manucol and antifoam (PBMA) and plated onto tryptic soy agar (TSA) plates that were incubated at 37º ± 2ºC for 24 hours.

A. brasiliensis (ATCC 16404) was grown on malt extract agar (MEA) (n=5) at 37° ± 2ºC for 48 hours. Spores were retrieved by pouring 10 ml of sterile distilled water onto each agar plate and then gently removing the growth, using a sterile L-shaped inoculating loop into a sterile container (50 ml), to achieve a spore suspension of 3.9 x 10^6 spores/ml^{-1}.

A stock culture (4ºC) of *B. diminuta* (NCIMB 11091) was grown in TSB (100 ml) in a shaking water bath (130 rpm, 30ºC for 19 hours ± 1 hour). The suspension was centrifuged at 2,000 g for 30 minutes. The pellet was washed three times, after which the supernatant was discarded and the cells re-suspended in sterile distilled water (60 ml) to achieve a suspension of 6 x 10^9 cfu/ml^{-1}.

HEPA Filters

Pre-packed hydrophobic pleated membrane HEPA filters (BB22-15, Pall, Portsmouth, UK) were aseptically dismantled. An area of the filter material (0.042 m in diameter) was removed and assembled in a sterile filter holder (16508 B, polycarbonate in-line 50-mm filter holder, Sartorius-stedim, France) which was connected to the Henderson Apparatus (Henderson, 1952) to challenge filters with the microbial aerosol (Druett, 1969).

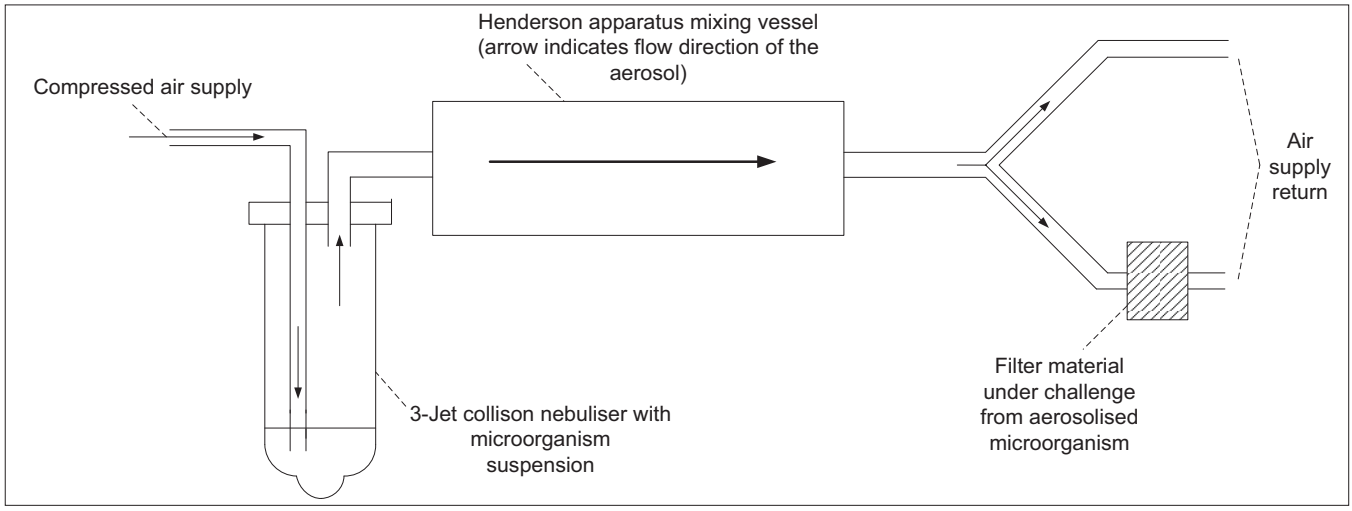
Individual suspensions of *B. atrophaeus, S. epidermidis,* MS-2 coliphage*, E. coli*, *B. diminuta*, and *A. brasiliensis* were placed into the Collison nebulizer (BGI, Inc., Waltham, MA) to generate an aerosol that passed along the flow channel of the Henderson Apparatus (l=77cm and d=5cm) and impacted with the filter. The contaminated filter holder was then removed from the Henderson Apparatus and connected to a clean vent filter (to prevent external contamination), and this combination was then fitted to a single ACI multistage centrifugal fan (6-stage MS8, Air Control Industries Ltd., Axminster, UK). The centrifugal fan unit was used to draw conditioned room air through both filters at a constant flow rate, as measured by a mass flow meter (TSI 4040, TSI, Shoreview, MN) giving a face velocity ranging from 0.4-0.5 m/s^{-1}. This apparatus was used to simulate HEPA filters mounted within a laboratory over a period of 6 days. Following exposure, a section from each filter (0.016 m in diameter) was removed for each microorganism (in triplicate) and placed into PBMA (10 ml) containing sterile glass beads (4 glass beads, each 4 mm in diameter) and vortexed (1 minute). Serial dilutions were then prepared and were plated onto the appropriate medium (TSA for *S. epidermidis*, *E. coli*, *B. diminuta*; *B. atrophaeus* and MEA for *A. brasiliensis*) and incubated as described above.

Complete pre-packed sterile filters (Maus et al., 2001) were also used in this study to determine the recovery of *B. atrophaeus* over a longer time period. The filter cartridge was connected to the Henderson Apparatus and a suspension of *B. atrophaeus* was placed into the Collison nebulizer to challenge the pleated membrane as described above for the filter holder (Figure 1). The filter cartridges (Maus et al., 2001) were then removed from the Henderson Apparatus and connected to an airflow apparatus operated at room temperature. A centrifugal fan (ACI, Axminster, UK) was used to blow air through a H14 HEPA filter into a central column, onto which the test filters were fitted. This allowed filtered air to be blown through each filter simultaneously at a constant rate (0.4-0.5 m/s^{-1} face velocity) for up to 210 days.

Filters were removed from the airflow apparatus and an area (0.016 m in diameter) of each filter material was aseptically removed from each monthly sample (in triplicate) and placed into PBMA (10 ml) containing sterile glass beads (4 glass beads, each 4 mm in diameter) and vortexed (1 minute). Each set of monthly filters were processed (in triplicate) and serial dilutions were plated onto TSA plates and incubated as described above.

Figure 1

Schematic of the microbial challenge system showing the Collision Nebulizer, Henderson apparatus, and filter assembly.



Plaque Assay

E. coli was sub-cultured from a TSA plate (37° ± 2°C for 20 hours) and transferred into sterile nutrient broth in a glass bottle, mixed and incubated (37° ± 2°C for 260 minutes). Soft phage agar was melted and held in a water bath at 60° ± 2°C. Prior to use it was allowed to cool to 40° ± 2°C for use in the assay. MS-2 suspension in PBMA (100 µl) and 60 µl of the *E. coli* suspension were added to the soft agar which was poured on a Tryptone Soya Broth Agar (TSBA) plate. Duplicate samples were prepared and incubated at 37° ± 2°C overnight. Viral plaques were counted the following day and data from plates containing 30 to 300 plaque forming units (PFU) were recorded.

The microbial recovery from the membrane filters was calculated by multiplying the cfu/ml^{-1} by the area of the membrane placed in the holder (1.38 x 10^{-3}/m^{-2}) and dividing by the area of the portion of the membrane from the holder used for assaying the amount of colonies (2.01 x 10^{-4}/m^{-3}) and by the area of whole membrane unit (0.07 m^{2}) in the case of *B. atrophaeus* in the 210-day study.

Results and Discussion

The results indicated that survival times were different for the range of microorganisms deposited onto the HEPA filter (Figures 2 and 3). *E. coli* and *B. diminuta* were not recoverable after 2 days whilst *S. epidermidis* was not recoverable after 6 days (Figure 2).

B. atrophaeus and *A. brasiliensis* showed no significant loss of viability on the HEPA filters for up to 6 days (Figure 2) whilst MS-2 coliphage lost less than 1 log (80% of original sample) over 6 days. The additional experiments using the filter cartridges demonstrated that *B. atrophaeus* spores survived for 210 days with little loss in viability (Figure 3).

The results for *A. brasiliensis* and MS-2 coliphage (used as a viral surrogate) were comparable with *B. atrophaeus*. *A. brasiliensis* spores are known for their resistance to desiccation (Bearg, 1993) and MS-2 virus, which is a non-enveloped virus, lacks a lipid bilayer, and has been shown to survive longer than enveloped virus (Rengasamy et al., 2010; Rutala & Weber, 2004) such as influenza.

These findings support other studies demonstrating that *B. atrophaeus* survives longer than *S. epidermidis* (Brosseau, 1994) on filters. Not surprisingly, increased survival of *B. atrophaeus* has been demonstrated on filters in the presence of nutrients (Wang et al., 1999). Whilst other studies have also demonstrated the survival of *B. atrophaeus* for up to 5 days (Maus et al., 2001) and *A. brasiliensis* for several weeks under air flow conditions on filters (Kemp et al., 2001), this current study demonstrated that *B. atrophaeus* can also survive on filters (with air flow) for more than 210 days (Figure 3) in the absence of contaminating nutrients. In addition, bacteriophage MS-2 was also capable of retaining viability for up to 7 days on filter surfaces and so may indicate that other viruses could also survive.

Figure 2

Survival of *B. atrophaeus*, MS-2 coliphage, *A. brasiliensis*, *S. epidermidis*, *E. coli*, and *B. diminuta* on the filter membrane over 6 days (bars represent means of triplicate counts and error bars represent standard deviations).

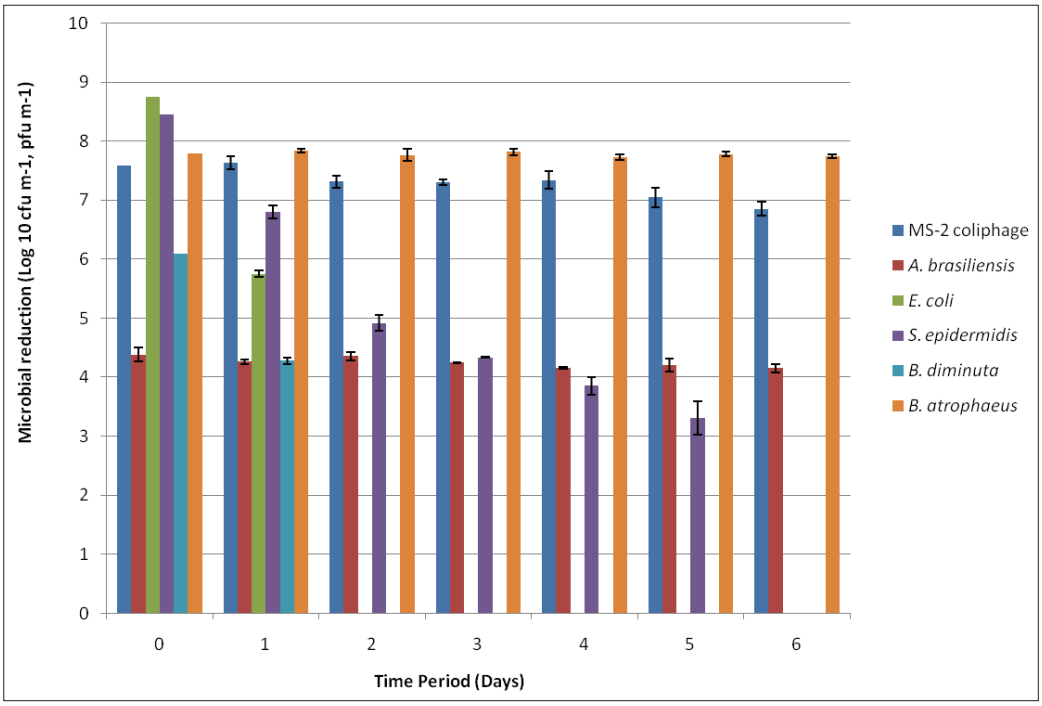
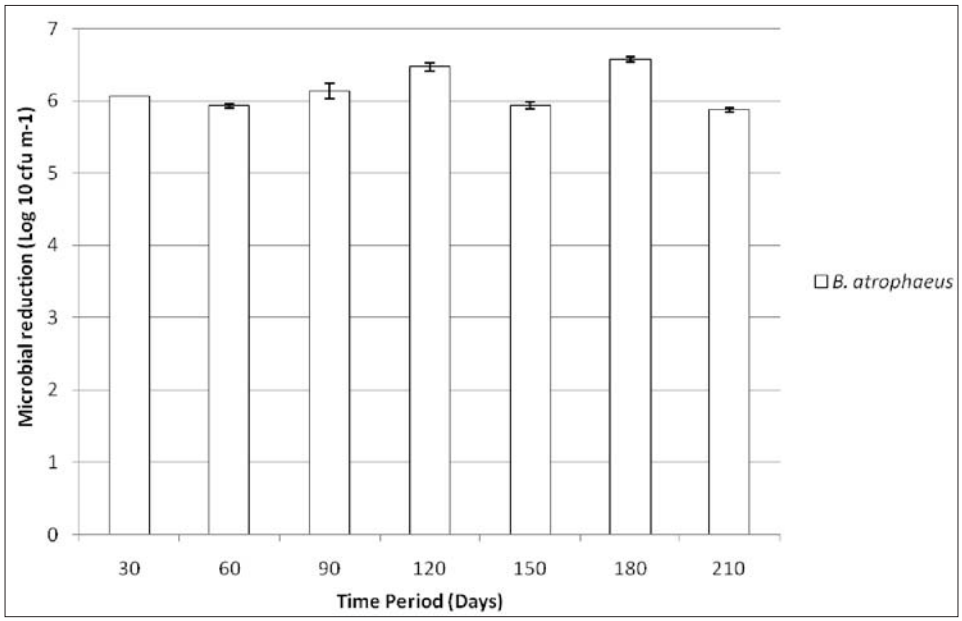


Figure 3

Survival of *B. atrophaeus* for 210 days on filter membranes (bars represent means of triplicate counts and error bars represent standard deviations).



Whilst this study used a range of non-pathogenic microorganisms, it would be prudent to be cautious with HEPA filters removed from laboratories where pathogenic microorganisms are used, as this removal process may indeed present a situation where occupational exposure could occur. A risk assessment should be conducted to determine potential occupational exposure hazards that may be present during removal and disposal of filters. Based on the risk assessment, mitigation measures may involve decontamination of filters, use of personal protective equipment, or other methodologies prior to removal and disposal of HEPA filters.

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