



FIGURE. Schematic representation of the test apparatus used for aerosol challenges.

Removal of *Mycobacterium* Species by Breathing Circuit Filters

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ABSTRACT

Breathing circuit filters (BCFs) are used in respiratory and critical-care settings for humidification of air and to limit transmission of respiratory pathogens. Three types of BCFs (Pall BB 25A, BB 100, and HME 15-22) were evaluated (in triplicate) for removal of *Mycobacterium* species. Filters were challenged with aerosolized *Mycobacterium bovis* (a surrogate for *Mycobacterium tuberculosis*), at minimum total concentrations of 10^4 colony-forming units. No *M bovis* was recovered in the effluent, providing removal efficiencies of >99.99% to >99.999% for the filters tested (*Infect Control Hosp Epidemiol* 1997;18:252-254).

Medical devices used for respiratory intervention, by their very nature, carry a risk of nosocomial infection because they bypass normal host defense barriers. The National Nosocomial Infections Surveillance (NNIS) System has indicated that nosocomial pneumonia is the leading cause of hospital-acquired infection,¹ accounting for approximately 13% to 18% of all nosocomial infections in the United States. Rates of pneumonia are 7- to 21-fold higher in intubated patients as compared to patients without a respiratory therapy device.²

Tuberculosis is resurging, adding to the plethora of existing respiratory infections. Globally, the annual mortality rate for tuberculosis has been estimated at 3 million.³ Outbreaks of multidrug-resistant strains, along with the

enhanced vulnerability of human immunodeficiency virus (HIV)-positive individuals to tubercle bacilli,⁴ combined with socioeconomic factors, have contributed to the reemergence of tuberculosis.

Heat and moisture exchanger (HME) devices used in respiratory- and critical-care settings serve a dual function: humidification of air and prevention of contamination of breathing apparatus, thus limiting cross-infection between patients. The effectiveness of the breathing circuit filters (BCFs) evaluated in this study has been documented adequately.⁵⁻⁸ Based on a clinical study, Gallagher et al⁵ concluded that the use of BCFs obviated the need for sterilization of breathing circuits or decontamination of ventilators. Considerable data exists demonstrating the efficiency of BCFs for removal of monodispersed challenge bacteria or viruses.⁶⁻⁸ Recognizing the concerns over the iatrogenic dissemination of *Mycobacterium* species, the present study was undertaken to evaluate the efficiency of removal of aerosolized *Mycobacterium bovis* (a surrogate for *Mycobacterium tuberculosis*) by three types of breathing circuit filters (Pall BB 25A, BB 100, and HME 15-22).

METHODS

Filters

Three types of breathing circuit filters (Pall BB 25A, BB 100, and HME 15-22; Pall Biomedical Products Co, East Hills, NY) were evaluated. All three BCFs are bidirectional hydrophobic membrane filters that are impervious to bodily fluids and aerosolized droplets, while simultaneously serving a moisture exchange function. Greater than 99.999% bacterial and viral removal efficiency has been reported for these filters.⁶⁻⁸ Modifications in their internal volume and resistance-to-flow parameters make the filters suitable for various niches: the BB 25A, anesthesia; the BB 100, respiratory- or critical-care environments; the HME 15-22, both anesthesia and respiratory- or critical-care settings.

Microorganism and Growth Conditions

The challenge microorganism, *M bovis* ATCC 35746, was grown by the method recommended for evaluation of tuberculocidal activity of disinfectants.⁹ For each aerosol challenge, 5 mL of a suspension containing a minimum of 1.5×10^6 /mL was loaded into the nebulizer. *M bovis* was titered on Middlebrook 7H11 agar by the membrane fil-

tration method, using 0.45 μm -rated membranes as the recovery membrane. All plates were incubated at 37°C for 3 to 6 weeks. Following incubation, plates were scored for the numbers of *M bovis*. Results are reported as total colony-forming units (CFU) upstream and downstream of the test filter.

Aerosol Challenge Experiments

Aerosol challenges were conducted using the aerosol challenge apparatus diagrammed in the Figure. Essentially, the test set-up is composed of the nebulizer, the mixing chamber, and a split-stream sampling system. An aerosol of *M bovis* ATCC 35746 was generated using a nebulizer (DeVilbiss Model 40, DeVilbiss Co, Somerset, PA). The pressure for nebulization was adjusted to 9 to 9.5 psi. The microbial aerosol was introduced into the mixing chamber (a pressure vessel of 2-gallon capacity), along with compressed dry air (<-50 dew point) that had been sterilized by passage through an 0.2 μm -rated filter at a flow rate of 1 cubic ft per minute (28.3 liters per minute [lpm]). The aerosol leaving the mixing chamber passed through an evaporator column that ensured that the microbial challenge to the filter was delivered as a dry aerosol rather than as microdroplets.¹⁰ Sampling was done using a vacuum switcher device (which alternated, between the upstream and the downstream impingers, at 30-second intervals) and a split-stream liquid impingement method. Sets of two 14 \pm 0.2 lpm impingers (Ace Glass, Vineland, NJ), attached in tandem, each containing 20 mL of phosphate buffer (1 M, pH, 7.2), were positioned upstream and downstream of the test filter to ascertain concurrently the actual challenge to the filter and the removal efficiency of the filter, respectively. The test was run for a total of 6 minutes, followed by a 2-minute air flush, to ensure that the system had been purged of the aerosol. Any excess airflow not collected by the impingers was vented through an exhaust filter located upstream of the filter.

Each of the types of breathing circuit filters (BB 25A, BB 100, and HME 15-22) was challenged in triplicate, with each individual filter tested twice. Following challenge, the buffer from the impingers located upstream and downstream of the test filters was assayed. This allowed for precise determination of the actual challenge level for each test filter and calculation of the efficiency of titer reduction of the input challenge level. Additionally, titers were performed on the challenge suspension prenebulization and postnebulization to ensure that nebulization did not alter the viability of the microorganism.

The efficiency of the filter was evaluated in terms of the log reduction value (LRV)=

$$\text{LRV} = \log_{10} \frac{\text{Total number of organisms in the challenge suspension}}{\text{Total number of organisms in filtrate}}$$

When the filtrate was sterile, a nominal value of 1 was used as the number recovered and the results expressed as greater than the calculated value.

The ratio of the difference between the numbers of challenge microorganism recovered upstream and down-

TABLE
RETENTION OF *MYCOBACTERIUM BOVIS* BY BREATHING
CIRCUIT FILTERS

Filter Type	Total Challenge (CFU)	Total Recovery (CFU)	Log Reduction Value	Removal Efficiency (%)
Pall BB-25A*	1.4 \times 10 ⁴	0	>4.14	>99.993
Pall BB-100†	9.9 \times 10 ⁴	0	>5.0	>99.999
Pall HME 15-22‡	2.5 \times 10 ⁴	0	>4.4	>99.996

Abbreviation: CFU, colony-forming units.

* Lot No. 005310.

† Lot No. 205374.

‡ Lot No. 28140.

stream of the test filter to the average total challenge received by the filter provides an indication of the removal efficiency of the filter:

$$\text{Removal efficiency (\%)} = \frac{\text{Average total challenge} - \text{Average total recovery}}{\text{Average total challenge}} \times 100$$

RESULTS

Results for the three types of BCFs are shown in the Table. When total challenge levels of 1.1 \times 10⁴ to 1.9 \times 10⁴, 1.7 \times 10⁴ to 2.1 \times 10⁵, and 1.6 \times 10⁴ to 3.7 \times 10⁴ CFU were used to challenge BB 25A, BB 100, and HME 15-22 filters, respectively, in all cases, no *M bovis* was recovered downstream of the test filters. Log reduction values of 4 to 5 logs were demonstrated. Removal efficiencies ranged from >99.99% to >99.999%.

DISCUSSION

In view of the resurgence of tuberculosis and the distinct threat of cross-infection by contaminated mechanical devices, this study was undertaken specifically to document the removal of *Mycobacterium* species by BCFs. Results are as expected: total retention of *M bovis*. The predicted retention is based on minimum removal efficiencies of 99.999% for *Brevundimonas (Pseudomonas) diminuta*,^{7,8} which is a smaller bacillus than *M bovis*. Logically, therefore, *Mycobacterium* species, which are bacilli ranging from 0.2-0.7 μm \times 1.0 to 10 μm in size,¹¹ should be removed.

The pathogenic potential of aerosolized *M tuberculosis* dictated the use of *M bovis* as the challenge organism. The use of *M bovis* as a surrogate for *M tuberculosis* has been documented extensively on the basis of genetic studies,¹² numerical taxonomy,¹³ and in-vitro and in-vivo immunologic studies.^{14,15} Also, *M bovis* ATCC 35743 is recommended by the Association of Official Analytical Chemists as the indicator organism for the assessment of the tuberculocidal activity of disinfectants.⁹

The data reported herein demonstrate the efficient removal of *Mycobacterium* species by three different types of breathing circuit filters: the Pall BB 25A, BB 100, and HME 15-22. It should be noted that these results are conservative, because the LRV and the percent removal efficiency are lim-

ited by the concentration of *M bovis* in the input aerosol challenge. Filter retention efficiency possibly was higher than reported, given that no *M bovis* was detected downstream of the test filters. In conjunction with the considerable documentation of microbial removal by these filters^{6,8} and the demonstration of clinical effectiveness in maintaining circuit cleanliness,⁵ the data presented here provide further evidence that these breathing circuit filters would limit transmission or spread (to equipment) of microbial pathogens and suggest that these filters could provide protection against the transmission of *Mycobacterium* species in a respiratory-care setting.

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