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An In Vivo *Evaluation of the Mycobacterial Filtration Efficacy of Three Breathing Filters Used in Anesthesia*

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Background: **The use of breathing filters (BFs) has been recommended to protect the anesthesia apparatus in proven or suspected cases of tuberculosis. Some investigators have also suggested the use of BF to alleviate the need to change anesthesia breathing circuits after each case. This study evaluated the filtration efficacy of three different BFs to prevent mycobacterial contamination of breathing circuits in a model that uses a test animal.**

Methods: **Ten Pall BB25A® (pleated hydrophobic) (Pall Canada Ltd., Mississauga, Ontario, Canada), six DAR Barrierbac S® (felted electrostatic; Mallinckrodt DAR, Mirandola, Italy), and six Baxter Airlife® (felted electrostatic; Baxter Canada, Mississauga, Ontario, Canada) BFs were studied. For each BF tested, 20 ml of a high concentration suspension of *Mycobacterium chelonae* (range, 2.0×10^7 to 9.0×10^7 colony-forming units/ml) was nebulized during 2 h at the proximal end of the endotracheal tube of anesthetized pigs. At the end of the nebulization period, the BFs were sampled for culture. The titer reduction value (number of microorganisms challenging the BF divided by the number of microorganisms recovered downstream of the BF) and the removal efficiency (difference between the number of microorganisms challenging the BF and the number of microorganisms recovered downstream of the BF, divided by the number of microorganisms challenging the BF) were calculated.**

Results: **The median titer reduction values were 5.6×10^5, 6.0×10^5, and 8.0×10^8 ($P < 0.0005$), and the median removal efficiencies were greater than 99.999%, greater than 99.999%, and 100% (P = not significant) for the DAR Barrierbac S®, the Baxter Airlife®, and the Pall BB25A®, respectively.**

Conclusions: **Among the three BFs studied, only the Pall BB25A® completely prevented the passage of *M. chelonae*, thus protecting the anesthesia breathing circuit from mycobacterial contamination.**

CONTAMINATED anesthesia equipment has been implicated as a causative factor of postoperative pulmonary infections, and microorganisms have been isolated in almost every part of the anesthesia breathing system.[1–3] Therefore, the current recommendations of both the Centers for Disease Control and the American Society of Anesthesiologists state that a sterile (or alternatively submitted to high-level disinfection) anesthesia breathing circuits should be used for every patient.[3,4] In proven or suspected cases of tuberculosis, the Centers for Disease Control recommend that a breathing filter (BF) be placed between the anesthesia equipment and the patient's airway.[5] The use of a BF placed between the Y-piece of the anesthesia breathing circuit and the endotracheal tube has also been proposed to prevent contamination of the breathing circuit. This could allow the reuse of the same breathing circuit for several patients, and as long as the BF is less expensive than the breathing circuit, this practice would be cost efficient.[6,7] Although tuberculosis prevalence has slightly decreased in recent years (after a surge in the late 1980s and early 1990s), it remains a serious infectious threat, even more with the emergence of multiresistant *Mycobacterium tuberculosis* strains.[8] Furthermore, nosocomial transmission of tuberculosis to both patients and healthcare workers has been reported.[9,10] Many BFs are available in North America, built with different materials and relying on different filtration mechanisms. Their performance for bacterial filtration has been investigated both in laboratory studies and in the clinical setting. However, to our knowledge, no study has evaluated the efficacy of BFs against *M. tuberculosis* in the clinical setting.

Because of the high contagious potential of *M. tuberculosis*, mandating a level 3 experimental facility, clinical studies are difficult to conduct. Therefore, other mycobacteria with lower pathogenicity have been used as surrogates for *M. tuberculosis*.[11] The objective of this study was to evaluate the mycobacterial filtration efficacy against *Mycobacterium chelonae* of three different BFs available in North America in an animal model designed to reproduce the clinical setting. The second objective was to explain the performance of the three filters by submitting them to scanning electron microscopy.

Materials and Methods

The different models of BF used in anesthesia in the province of Quebec were identified by an informal survey. Representatives from manufacturers of BFs were also approached and asked to submit a proposal of a model for a BF. These BFs had to present the following characteristics: an *in vitro* bacterial filtration efficiency greater than 99.9999% and a small size and low dead space volume compatible with use in the clinical setting. Three different anesthesia BFs of different construction were selected for inclusion in this study. Six samples of

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the DAR Barrierbac S® (Mallinckrodt DAR, Mirandola, Italy), six samples of the Pall BB25A® (Pall Canada Ltd., Mississauga, Ontario, Canada), six samples of the Baxter Airlife® (Baxter Canada, Mississauga, Ontario, Canada), and four more samples of the Pall BB25A® were sequentially tested. The Pall BB25A® is made of a pleated paper customized with fiberglass and ceramic. It possesses naturally induced electrostatic charges and has hydrophobic and hygroscopic properties. The DAR Barrierbac S® is made of felted polypropylene fibers set up in multiple layers as a mat. The polypropylene fibers are externally electrostatically charged and have hydrophobic properties. The filter media is also treated with calcium chloride to gain hygroscopic properties. The Baxter Airlife® is also made of felted polypropylene fibers set up in multiple layers as a mat. The polypropylene fibers are externally electrostatically charged and have hydrophobic properties, but no hygroscopic property is claimed by the manufacturer. The three models of BF are presented in a sterile package.

Preparation of M. chelonae

The strain selected was isolated repeatedly from the sputum of an immunosuppressed patient, and its identification was confirmed by the Quebec Public Health Laboratory (Sainte-Anne-de-Bellevue, Quebec, Canada). The day before each experiment, a saline suspension equivalent to a 1.0 MacFarland standard was obtained from a 48-h old subculture from a blood agar plate. After sampling for determination of the exact concentration of *M. chelonae*, the suspensions were prepared in 20-ml aliquots in sterile test tubes and refrigerated until use.

Experimental Procedures

The protocol conformed to the Canadian Council of Animal Care's Code of Ethics and was approved by the Animal Care Committee of Laval University (Laval University, Quebec City, Quebec, Canada). The study was conducted at the Experimental Medicine Laboratory of Laval University. The animals used were healthy adult female pigs weighing 75–100 kg that were concurrently used in an evaluation of surgical implants. These animals had to be anesthetized either to implant or to remove a surgical mesh in their abdominal wall. The animals were fasted overnight and received an intramuscular premedication of acepromazine, atropine, butorphanol, and midazolam. An intravenous line was inserted, and anesthesia was induced with thiopental (1–4 mg/kg). After the airway was topically anesthetized with 10% lidocaine, the trachea was intubated with a sterile endotracheal tube during spontaneous ventilation. The lungs were ventilated with 100% oxygen at a rate of 10–12 min^{-1} with a tidal volume of 8–10 ml/kg. Anesthesia was maintained with intravenous morphine, midazolam, and pancuronium. Hydration was provided with normal saline at a rate of 12–15 $ml \cdot kg^{-1} \cdot h^{-1}$. Monitoring included electrocardiography, noninvasive blood pressure monitoring, pulse oximetry, and airway manometry. At the end of the procedure, depending on the needs of the surgical implant protocol, the animals were either killed with a high dose of thiopental (125 mg/kg) or woken up after muscle relaxant reversal.

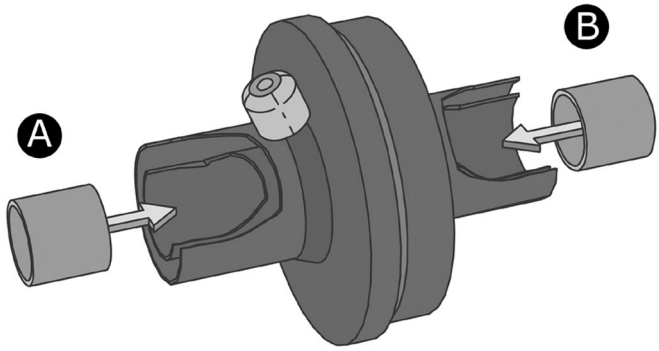

Fig. 1. Drawing of a breathing filter showing the two 15-mm-long sterile polyvinyl chloride tubes inserted into the connectors of the breathing filter ([*A*] circuit side of the breathing filter, [*B*] animal side of the breathing filter). These polyvinyl chloride tubes served as physical support for the microbiologic sampling.

For each experiment, a new sterile disposable clear anesthesia breathing circuit of 22 mm in diameter and 183 cm in length (Trudell Medical Ltd., London, Ontario, Canada) and a new sterile anesthesia BF were used. A 15-mm-long sterile polyvinyl chloride (PVC) tube with a 15-mm external diameter was inserted into each of the two connectors (animal and circuit sides) of the BF tested (fig. 1) These PVC tubes served as physical support for the microbiologic sampling at the end of the experiment. After induction of anesthesia, the BF tested was inserted aseptically between the endotracheal tube and the Y-piece of the anesthesia breathing circuit. An Up-Draft II Neb-u-mist® nebulizer (Hudson Respiratory Care Inc., Temecula, CA) was inserted between the BF and the endotracheal tube. Twenty milliliters of the suspension containing *M. chelonae* (concentration range, 2.0×10^7 to 9.0×10^7 colony-forming units [cfu]/ml) was nebulized continuously with a carrier flow of 6 l/min over 120 min. This setup intended to reproduce the excretion of a high density of mycobacteria by an infected animal. At the end of the nebulization period, the BF was removed from the breathing circuit, and the two PVC tubes were extracted from the BF connectors under sterile conditions. Both PVC tubes were soaked separately in test tubes containing 15 ml brain–heart infusion broth and were sent to the microbiologic laboratory within 1 h.

Controls

To rule out external contamination, control cultures and experiments were performed. First, sampling of the exterior surface of the BF connectors (animal side and circuit side) was performed twice during the study. The

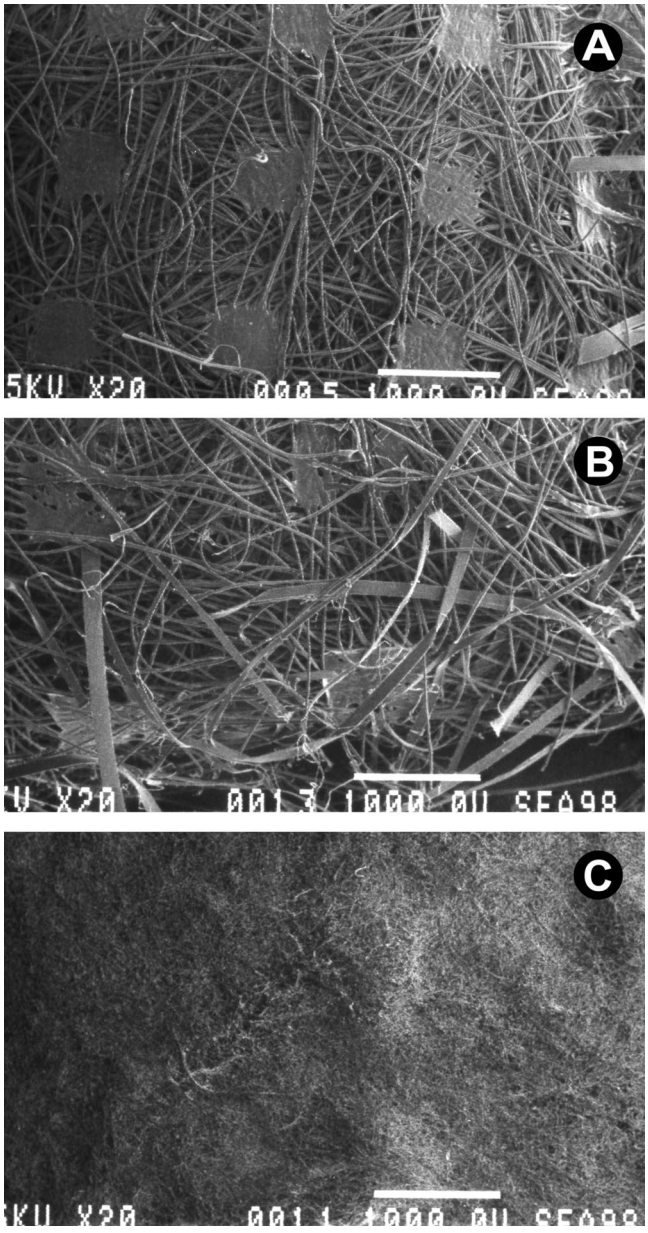

Fig. 2. Scanning electron microscopy (20× magnification) of the clean filtering membrane of the three breathing filters tested: DAR Barrierbac S® (Mallinckrodt DAR, Mirandola, Italy) (*A*), Baxter Airlife® (Baxter Canada, Mississauga, Ontario, Canada) (*B*), and Pall BB25A® (Pall Canada Ltd., Mississauga, Ontario, Canada) (*C*). The filtering membrane of the Pall BB25A® is much more compact and its pore size are much smaller.

purpose of this control was to eliminate the possibility of an external contamination source. Second, the experimental procedure was run in two cases with the animal anesthetized, but with nebulization of a solution free of *M. chelonae*. This was done to eliminate the possibility of back flow contamination from the anesthesia machine.

Laboratory Processing

The test tubes were first submitted to ultrasound (55,000 Hz, 125 W) for 15 s to dislodge mycobacteria from the PVC tubes. Then, 1 ml brain–heart infusion broth was inoculated in a BACTEC® system (BD Diagnostic Systems, Sparks, MD) and incubated for 6 weeks for semiquantitative analysis. One milliliter of the brain–heart infusion broth was also serially diluted in 8 test tubes containing 9 ml saline each and was vortexed. One hundred microliters of these suspensions was then plated on chocolate blood agar and on Thayer-Martin agar. The inoculated plates were incubated at 35°C in a 5% CO_2–enriched atmosphere for up to 2 weeks. Plates were examined at 48-h intervals. Colonies were Gram and Ziehl stained to confirm the presence of mycobacteria, and the mycobacterial count was calculated from the serial dilution of the original inoculum. Laboratory personnel were blinded to which filter had been used.

Data Analysis

The filtration efficacy of the BF was determined by the titer reduction value (TRV) and the removal efficiency (RE). TRV is calculated by dividing the total number of microorganisms challenging the BF (20 ml of the nebulized suspension) by the number of microorganisms recovered downstream of the BF (total number recovered on the PVC tube on the circuit side). When the circuit side culture was negative for *M. chelonae*, a nominal value of 1 was used as the denominator.

$$\text{TRV} = \frac{\text{Total } \textit{M. chelonae} \text{ Challenge (cfu)}}{\text{Total } \textit{M. chelonae} \text{ Recovery (cfu)}}$$

RE is calculated by dividing the difference between the total challenge and the total recovery by the total challenge.

$$\text{RE} = \frac{\text{Total } \textit{M. chelonae} \text{ Challenge (cfu)} - \text{Total } \textit{M. chelonae} \text{ Recovery (cfu)}}{\text{Total } \textit{M. chelonae} \text{ Challenge (cfu)}} \times 100\ (\%)$$

Data are expressed as median with range. BFs were compared for TRV and RE using the Wilcoxon rank scores for unpaired data and the Kruskal-Wallis test. A P value less than 0.05 was considered significant.

Results

One DAR Barrierbac S® sample was accidentally damaged during laboratory handling. Data of the five remaining DAR Barrierbac S® BFs were included in the analysis. Experiments were performed on 8 different days. The 20 ml *M. chelonae* was nebulized over 2 h in all cases. The median number (range) of mycobacteria nebulized was 1.0×10^9 cfu (1.0×10^9 to 1.0×10^9) for the DAR Barrierbac S®, 1.0×10^9 cfu (8.0×10^8 to 1.8×10^9) for the Baxter Airlife®, and 8.0×10^8 cfu (4.0×10^8 to 1.8×10^9) for the Pall BB25A® (P = not significant; table 1) *M. chelonae* was recovered on the circuit side of the BF in all DAR Barrierbac S® and Baxter Airlife® BFs but not in

Table 1. Efficacy of the DAR Barrierbac S®, Baxter Airlife®, and Pall BB25A® Breathing Filters at the End of a 2-Hour Nebulization Period of a Standardized Load of *Mycobacterium chelonae*

	Total Mycobacterial Challenge,* cfu	Total Mycobacterial Recovery,† cfu	TRV	RE, %
DAR Barrierbac S®				
1	1.0×10^9	1.5×10^4	6.7×10^4	> 99.998
2	1.0×10^9	9.0×10^2	1.1×10^6	> 99.999
3	1.0×10^9	1.2×10^5	8.3×10^3	> 99.988
4	1.0×10^9	7.5×10^2	1.3×10^6	> 99.999
5	1.0×10^9	1.8×10^3	5.6×10^5	> 99.999
Baxter Airlife®				
1	8.0×10^8	1.1×10^4	7.6×10^4	> 99.998
2	8.0×10^8	1.5×10^3	5.3×10^5	> 99.999
3	1.0×10^9	6.0×10^2	1.7×10^6	> 99.999
4	1.0×10^9	2.3×10^4	4.4×10^4	> 99.997
5	1.0×10^9	1.5×10^3	6.7×10^5	> 99.999
6	1.8×10^9	9.0×10^2	2.0×10^6	> 99.999
Pall BB25A®				
1	1.6×10^9	nil	1.6×10^9	100
2	4.0×10^8	nil	4.0×10^8	100
3	1.8×10^9	nil	1.8×10^9	100
4	4.0×10^8	nil	4.0×10^8	100
5	4.0×10^8	nil	4.0×10^8	100
6	1.8×10^9	nil	1.8×10^9	100
7	8.0×10^8	nil	8.0×10^8	100
8	1.8×10^9	nil	1.8×10^9	100
9	4.0×10^8	nil	4.0×10^8	100
10	8.0×10^8	nil	8.0×10^8	100

When total recovery was negative (nil), the nominal value of 1 was used as the denominator in the titer reduction value (TRV) calculation. See text for statistical comparisons.

* Number of organisms nebulized on the animal side of the breathing filter. † Number of organisms recovered on the polyvinyl chloride tubing on the circuit side of the breathing filter.

cfu = colony forming units; RE = removal efficiency.

any of the Pall BB25A® BFs ($P < 0.0002$; table 1) The median (range) TRVs were 5.6×10^5 (8.3×10^3 to 1.3×10^6), 6.0×10^5 (4.4×10^4 to 2.0×10^6), and 8.0×10^8 (4.0×10^8 to 1.8×10^9) for the DAR Barrierbac S®, the Baxter Airlife®, and the Pall BB25A®, respectively ($P < 0.0005$; table 1). The median REs were greater than 99.999% for both the DAR Barrierbac S® and the Baxter Airlife®, whereas no *M. chelonae* was recovered on the circuit side of the 10 Pall BB25A® tested, yielding an RE of 100% (P = not significant; table 1).

Cultures of the exterior surface of the BF connectors were negative. Cultures on both the animal side and the circuit side of the two sham experiments were also negative. These negative results were confirmed by the BACTEC® system. This qualitative method uses a liquid medium that is highly sensitive for mycobacterial growth, with a low probability of false-negative results.[12] These media were incubated for a period of 6 weeks or until growth was detected. The BACTEC® system also confirmed all the negative cultures found on the circuit side of the Pall BB25A®.

Discussion

The main finding of this study is that the passage of *M. chelonae* was prevented only by one of the three BFs investigated. The TRV of the Pall BB25A® was also significantly better compared with the DAR Barrierbac S® and the Baxter Airlife®. REs were not statistically different for the three BFs, but it must be stressed that, in the clinical anesthesia setting, any mycobacterial passage should be considered as a failure of the BF. Therefore, our results suggest that the DAR Barrierbac S® and the Baxter Airlife® would not have reliably protected the anesthesia breathing circuit from mycobacterial contamination. Most studies on the mycobacterial filtration performance of BFs have been conducted by manufacturers in the laboratory using flow, pressure, and humidity conditions very different from those encountered in a clinical setting. Few have been reported in the peer-reviewed literature.[11] Moreover, although REs greater than 99.99% are reported by most manufacturers, BFs with different construction designs have not been compared between them in a clinical setting. As stated in a recent review, results of bench studies are not necessarily applicable to the clinical setting.[13]

In this study, the experimental design intended to reproduce as closely as possible the conditions usually encountered in the clinical setting. A standard anesthesia technique was used, and the equipment was similar to what is found in a standard operating room. Also, the anesthesia lasted long enough to reproduce the usual

time that a BF would have to protect the anesthesia circuit during the course of a normal operating schedule, although it cannot be ruled out that the performance of the BF might have been different had the exposure been longer than 2 h. Pigs have been used commonly for the study of pulmonary pathologies and were used here in the intent of reproducing as closely as possible the various conditions of ventilatory pressure and flow, temperature, and humidity encountered in a clinical setting.[14] *M. chelonae* was used because of its physicochemical characteristics similar to *M. tuberculosis*. The latter, because of its high contagious nature, necessitates stringent safety measures (level 3 facility), whereas the former is much less pathogenic, both for the investigator and for the animal, and is therefore acceptable to the animal care board.[15] Most studies on this topic have used low-pathogenic mycobacterium species, such as *M. chelonae* or *M. bovis*, as surrogates for *M. tuberculosis*.[11] The *M. chelonae* challenge was willingly chosen to be of a large magnitude to simulate the challenge presented by a patient with an active tuberculosis. Short lengths of PVC tubes were used to capture *M. chelonae* on both sides of the BF. This was a modification of a capture method commonly used in microbiology. The objective of this technique was to capture as many mycobacteria as possible. However, it is obvious that some mycobacteria adhered to structures other than the PVC tube, such as the breathing circuit, and this technique could have slightly underestimated the true number of mycobacteria. However, the same technique was used for all BFs, thus allowing for valid comparisons of mycobacterial recovery count, TRV, and RE. Although the animal side of the BF was also sampled, these data were not considered useful and were not included in the analysis. The total load of *M. chelonae* nebulized in the BF was rather used for calculation of TRV and RE according to standard methods.

The different efficacies of the three BFs tested can be explained by their design. The filtering membrane of the DAR Barrierbac S® and the Baxter Airlife® are made of felted polypropylene fibers arranged as a mat. They are not naturally hydrophobic and are called *felted electrostatic filters*. However, the filtering membrane of the Pall BB25A® is made of a pleated paper fibers bonded with fiberglass and ceramic. Filter membranes of this design have naturally occurring electrostatic charges, conferring hydrophobic properties, and are called *pleated hydrophobic filters*. More importantly, the Pall BB25A® pores are much smaller than those of the two other BFs (fig. 2). With such a design, water impermeability is obtained. It might not make much of a difference when the BFs are tested with a dry carrier gas, because small particles such as mycobacteria (size approximately 0.3 μm) are not only filtered by direct interception and inertial impaction but also undergo Brownian movement, which causes them to follow a convoluted pathway and gives them an effective diameter much larger than their real physical dimension.[13] However, in the clinical setting that was replicated by our protocol, the filtering must be done through the exhaled tidal volume, which is fully saturated with water. Under these circumstances, condensation frequently occurs in the endotracheal tube, the Y-piece connector, and the BF itself. This results in the accumulation of water particles of different sizes on the surface of the filtering media. Therefore, the ability to stop mycobacteria becomes dependent not only on the efficiency of the BF in dry gases but also on its capability to retain water that acts as a carrier for mycobacteria. Although they claim hydrophobic properties, the DAR Barrierbac S® and Baxter Airlife®, have large pores that allow water passage. They are submitted to an electromagnetic conditioning to gain their electrostatic capability, which can be lost when water penetrates the membrane.[16] With these two BFs, water was indeed frequently seen at the end of the protocol in the anesthesia breathing circuit but not with the Pall BB25A®. Important differences have been reported in performance against water penetration and microbial penetration between pleated hydrophobic filters and felted electrostatic filters.[17–19] Lloyd *et al.*[20] reported that the passage of hepatitis C virus in a humidified carrier gas was prevented by a pleated hydrophobic membrane but not by a felted electrostatic membrane. Hedley and Allt-Graham [21] also found that the DAR Barrierbac S® had an *in vitro* airborne bacterial filtration close to the Pall BB25A® but had a poor liquid-borne bacterial efficiency. These studies concur with the current one to suggest that the efficacy of a BF is determined by the type of filtering membrane and its pore size and by its hydrophobic characteristics. Because the felted polypropylene BFs have larger pores and lack natural hydrophobic properties, it can be suspected that, in the conditions encountered in clinical anesthesia, they are less effective in preventing mycobacterial contamination than the hydrophobic pleated paper BF. Finally, although the results of this study cannot be directly applied to all models of BF available on the market, other brands of BFs with similar construction design as the three BFs tested would be expected to behave similarly.

This has important implications both in the implementation of the current recommendations and in the future planning of strategies for infection control in anesthesia. The Centers for Disease Control recommend the use of a BF for every suspected or confirmed case of tuberculosis.[5] Our data show that two of the BFs studied cannot reliably protect the anesthesia breathing circuit and the soda lime canister from contamination by mycobacteria and thus should not be used for that purpose. It has also been suggested to use a BF between the Y-piece of the anesthesia breathing circuit and the proximal end of the endotracheal tube to avoid changing, sterilizing, or disinfecting the breathing circuit after each case and thus

decrease cost. However, some asymptomatic tuberculosis-infected patients unavoidably come undetected to the operating room, and the use of an ineffective BF might result in the contamination of the anesthesia breathing circuit and hence of the following patients.[22]

In conclusion, among the three BFs tested, only the Pall BB25A® completely prevented the passage of *M. chelonae* and protected the anesthesia circuit from mycobacterial contamination. We also conclude that BF built with felted polypropylene fibers might not protect the anesthesia breathing circuit from mycobacterial contamination in clinical conditions. Finally, this suggests that the water-retaining capability of a BF is an important feature in its ability to protect the anesthesia circuit from mycobacterial contamination.

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