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APPARATUS

The bacterial and viral filtration performance of breathing system filters*

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Summary

The bacterial and viral filtration performance of 12 breathing system filters was determined using test methods specified in the draft European standard for breathing system filters, BS EN 13328-1. All the filters were of two types, either pleated hydrophobic or electrostatic, and these two types differed in their filtration performance. The filtration performance is expressed in terms of the microbial penetration value, defined as the number of microbes passing through the filter per 10 million microbes in the challenge. The geometric mean (95% confidence limits) microbial penetration value was 1.0 (0.5, 3.5) and 2390 (617, 10 000) for the pleated hydrophobic and electrostatic filters, respectively, for the bacterial challenge, and 87 (48, 212) and 32 600 (10 900, 84 900), respectively, for the viral challenge. In general, there was little change in the microbial penetration values following 24 h simulated use. It is concluded that results from the tests specified in the draft standard will allow comparisons to be made between different manufacturers' products enabling an informed choice to be made.

Keywords *Infection*: bacterial; viral. *Equipment*: heat and moisture exchange filters.

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Cross-infection due to contaminated breathing systems has been known about for many years [1]. Following a suspected incident of cross-infection with hepatitis C in 1993 [2], breathing system filters are now recommended for use during anaesthesia to prevent cross-infection, unless the breathing system is replaced for each patient [3]. Wide variations in the filtration performance of different breathing system filters have been reported [4–9]. These studies used different test methods and organisms. Manufacturers also use a wide range of test methods [10]. Simple comparison between different studies may not, therefore, be valid. In general, filters are of two basic types: pleated hydrophobic or electrostatic. In a recent postal survey [11], the majority of respondents did not consider that the suggested increased filtration efficiency of pleated hydrophobic membrane filters outweighed the increased cost compared with electrostatic filters. The authors commented that acceptance of the draft European standard BS EN 13328-1 [12] should allow the efficiency of different types of breathing system filters to be compared objectively. We tested the bacterial and viral filtration efficiency of 12 breathing system filters according to the test methods specified in the draft standard.

Methods

The bacterial and viral filtration performance of the breathing system filters was determined both on unused devices and on 'conditioned' devices (conditioned by being subjected to 24 h simulated use). The 12 breathing system filters consisted of five pleated hydrophobic filters and seven electrostatic filters (Table 1).

Different samples of breathing system filters were used for the tests in an unused state and after conditioning. Conditioning was achieved by placing the breathing system filter in an apparatus, described in the draft

Table 1 Breathing system filters tested.

Manufacturer	Device	Type
Cory Bros	HEPA Filter/HME	Pleated hydrophobic
DAR	Sterivent Mini	Pleated hydrophobic
Pall	BB100	Pleated hydrophobic
Pall	BB25	Pleated hydrophobic
Portex	Thermovent HEPA	Pleated hydrophobic
Intersurgical	Clear-Therm	Electrostatic
Intersurgical	Filta-Therm	Electrostatic
Cory Bros	FilterVent	Electrostatic
DAR	Hygrobac 'S'	Electrostatic
Datex-Engström	HMEF1000	Electrostatic
Gibeck	Humid-Vent Filter Compact	Electrostatic
Ventalink	Ventalink Adult	Electrostatic

standard, which is based on that specified in the International Standard for heat and moisture exchangers, ISO 9360:1992 [13]. The apparatus was set to 'breathe' through the breathing system filter for 24 h with a tidal volume of 0.5 l, a frequency of 20 min^{-1} and an I : E ratio of 1 : 2, with an approximate square-wave flow. The 'expired' air had a relative humidity of $\geq 96\%$ at a temperature of 34 ± 1 °C; the 'inspired' air was dry at room temperature.

Prior to conditioning, the breathing system filter was weighed, and the pressure decrease across the device was measured at a gas flow of 30 $l.min^{-1.}$ The filter was then attached to the test apparatus, which was operated for 24 h. The filter was removed, reweighed and the pressure decrease across the device was measured again. The filter was then challenged with microbial aerosols, as described below, within 2 min of removal from the conditioning apparatus.

The draft standard specifies that the filtration performance of breathing system filters should be tested using microbes, as this technique is very sensitive over a wide range of performance levels.

Spores of *Bacillus subtilis* var. niger (NCTC 10073, ATCC 9372) were chosen as the bacterial model for the draft standard because they are nonpathogenic and are known to survive the stresses caused by aerosolisation [14]. On plating, *B. subtilis* var. niger also produces distinctive orange colonies that can be counted easily. *B. subtilis* var. niger is of a similar size to pathogenic bacteria, or smaller (Table 2).

MS-2 coliphage (NCIMB 10108, ATCC 15597-B1) was chosen as the viral model for the draft standard. This is an unenveloped single-stranded RNA coliphage, and is smaller than most human viruses (Table 2). MS-2 is also known to survive the stresses caused by aerosolisation [15].

As both microbes chosen are robust, and can survive the stresses caused by aerosolisation, the requisite number of microbes can be delivered to the filter under test over a short period.

Table 2 Comparison of the size of microbes used in this study with pathogenic microbes.

Microbe	Size (μm)
Bacillus subtilis var. niger	0.96–1.25 × 0.55–0.67
Pseudomonas aeruginosa	0.6 × 2
Tubercle bacilli	0.4 × 3
Staphylococci	≈ 1 × ≈ 1
Streptococcus pneumoniae	0.5 × 1
MS-2	0.023 (diameter)
Hepatitis B	0.042 (diameter)
Hepatitis C	0.03–0.06 (diameter)
HIV	0.08–0.1 (diameter)

The test procedure specified in the draft standard [12] was followed closely. The mobile version of the Henderson apparatus was used to deliver the challenge to the breathing system filter under test (Fig. 1) [16, 17]. A background circulation of air was generated through the apparatus in the direction shown and a microbial challenge from a nebuliser was added to this flow. Initially, valve L was open and clips V were closed. From the compressor-vacuum pump, N, the flow of air was directed through a proportioning valve, P, which controlled the relative flow through the humidifier, R, and the drier, S, to stabilise the humidity in the spray tube, G, to at least 96% RH, measured using the wet and dry thermometers, H. Compressed air (5 $l.min^{-1}$), A, measured by flowmeter U, was directed through the upper solenoid-operated valve, C, by the switch, B, and then through a three-jet Collison spray [18] containing distilled water, E, while the humidity of the circulating air stabilised. The total flow of air in the spray tube was maintained at 60 $l.min^{-1}$, measured by the flowmeters T and U, so that the flow through T was 55 $l.min^{-1}$ and the spill through O was 5 $l.min^{-1}$. When the humidity had stabilised, valve L was closed and clips V were opened so that the air leaving the spray tube was directed to a Y-piece. Tubes of equal length were connected between the Y-piece and the Porton all-glass-impingers J and K [19]. The filter under test, I, was attached to one tube so that the air flowed through the filter in the expiratory direction. Both impingers contained a critical orifice, which limited flow to 30 $l.min^{-1}$ when a gauge pressure of at least − 45 kPa downstream of the impingers was created by the pump, N. The air in the spray tube was maintained at a slightly negative pressure, to prevent microbes entering the ambient air in case there were any leaks. After 30 s, switch B was changed so that the compressed air was directed through the three-jet Collison spray nebuliser, F, containing the *B. subtilis* microbial suspension in distilled water for a maximum period of 1 min. Impinger K, therefore, collected microbes to determine the challenge

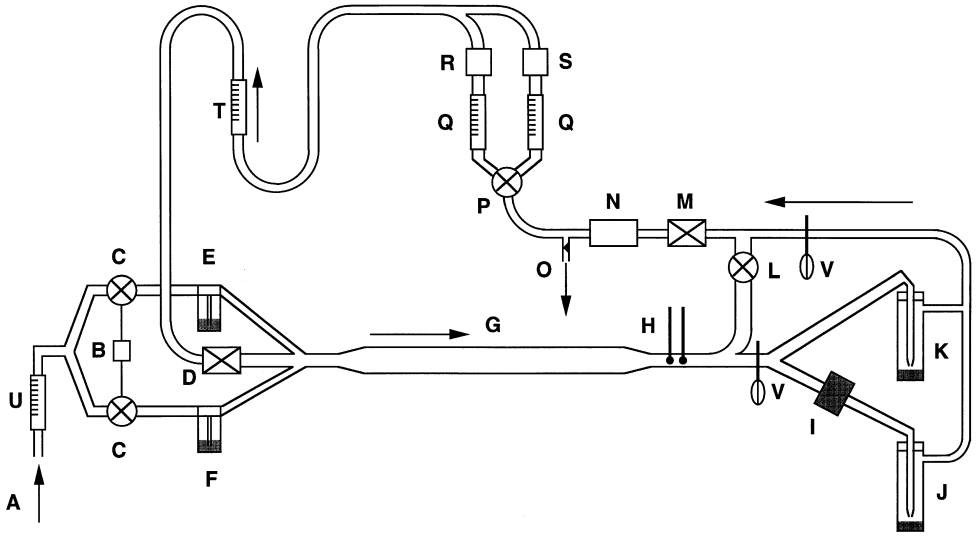

Figure 1 Test arrangement: Henderson apparatus [16, 17]. A, compressed air; B, two-way switch; C, solenoid-operated valve; D, filter; E, Collison spray containing distilled water; F, Collison spray containing challenge micro-organisms; G, spray tube; H, wet and dry thermometers; I, breathing system filter to be tested; J, downstream impinger; K, upstream impinger; L, valve; M, filter; N, compressor-vacuum pump; O, controlled gas leak; P, proportioning valve; Q, flowmeters: R, humidifier; S, drier; T and U, flowmeters; V, clips. Arrows show the direction of flow.

to the filter, and the impinger J collected microbes that had passed through the filter. The challenge to each filter was over 10^7 *B. subtilis* spores. After the test, the liquid in the impingers was transferred to labelled universal containers.

The filter was then challenged with MS-2. This was carried out by using another three-jet Collison spray containing MS-2 in 50% nutrient broth and fresh all-glass impingers and sterile connectors. The challenge to each filter was over 10^9 MS-2 phage. Assays of the liquids from the impingers were normally carried out within 4 h for both *B. subtilis* and MS-2.

As specified in the draft standard, the distribution of sizes of the particles containing the challenge micro-organisms in this system was determined using an Andersen sampler [20].

Each of the four combinations (of bacterial and viral test with before and after conditioning) was performed on three different samples of each breathing system filter giving a total of 12 results for each filter.

Results were expressed in terms of the microbial penetration value (MPV), defined as the number of microbes passing through the filter per 10 million microbes in the challenge, i.e.

$$\text{MPV} = (\text{number of microbes passing through the filter})/(\text{number of microbes in the challenge}) \times 10^{7}.$$

Statistical analysis

The Mann–Whitney test for two independent samples was used for analysis when comparing the results from two different groups of filters (Statview 4, Abacus Concepts Inc., Berkeley, CA). The Wilcoxon signed rank test for matched pairs was used for analysis when comparing the results from unused and conditioned breathing system filters. A p-value < 0.05 was considered significant. Because of the very wide range of MPVs, all calculations for statistical significance were carried out on $\log_{10}$(MPV), so results are expressed as geometric mean with asymmetric confidence limits.

Results

The distribution of aerosol particle size using the Andersen sampler showed that 80% or more of the challenge particles containing *B. subtilis* spores and MS-2 coliphage were < 2.1 μm in diameter, as required by the standard.

The mean (SD) bacterial and viral challenges were 3.52 (0.60) $\times$ 10^7 (range: 2.40–5.00 $\times$ 10^7) and 8.59 (2.82) $\times$ 10^9 (range: 4.85–16.6 $\times$ 10^9), respectively. The bacterial and viral challenges were, therefore, greater than the minimum levels specified in the draft standard (10^7 and 10^8, respectively).

The limits of detection of the test system are 2 colony-forming units (c.f.u.) for the bacterial challenge and 2 plaque-forming units (p.f.u.) for the viral challenge,

corresponding to a limit of detection of ≈0.5 for bacterial MPV and ≈0.005 for viral MPV (because of the greater number of microbes in the viral challenge).

The MPV values ranged from almost 0 to 183 000 and are displayed in terms of $\log_{10}$(MPV) in Fig. 2 with geometric means for each filter for each of the four combinations of conditions in Table 3. The geometric means and confidence limits for each type of filter, pleated hydrophobic or electrostatic, and each type of test were calculated (Table 4). The electrostatic filters were much less efficient than the pleated hydrophobic filters (right-hand column of Table 4), allowing over 2000 times as many bacteria and ≈400 times as many viruses through ($p < 0.0001$ for all four combinations of conditions). However, there was a wide variation in the performance of electrostatic filters, with the two devices manufactured by Intersurgical having lower MPVs than other electrostatic filters, in particular, the Filta-Therm (Fig. 2).

The MPVs from both types of breathing system filter were greater for the viral challenge than for the bacterial challenge (bottom row of Table 4): ≈90 times greater for the pleated hydrophobic filters ($p < 0.0001$) and ≈14 times for the electrostatic filters ($p = 0.0001$).

Paired comparisons were made between unused and conditioned filters for the bacterial challenge and also for the viral challenge, using the geometric means for each filter in Table 3. When the data for all filters were combined, there were no significant differences between

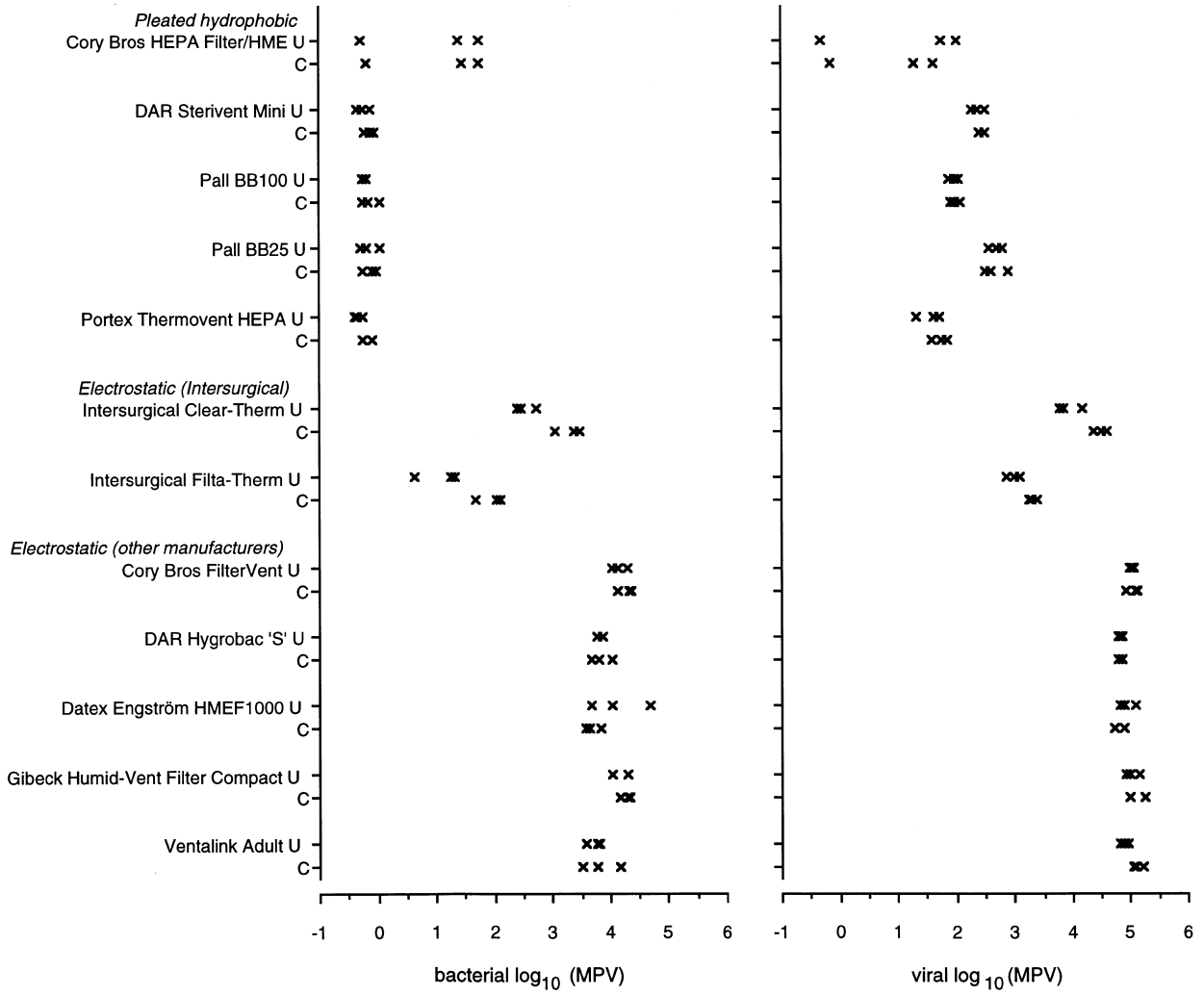

Figure 2 Bacterial and viral $\log_{10}$(MPV) for the breathing system filters tested. MPV is the microbial penetration value, which is the number of microbes passing through the breathing system filter per 10^7 microbes in the challenge. U, unused; C, conditioned (tested after 24 h simulated use). 'x' is the result from one test on each sample of each breathing system filter, with repeat samples for each type of test.

Table 3 Geometric means of the microbial penetration values (MPV) for bacterial and viral challenges both before use ('unused') and after 24 h simulated use ('conditioned').

	MPV (geometric mean, $n = 3$) Bacterial		Viral	
Device	**Unused**	**Conditioned**	**Unused**	**Conditioned**
Pleated hydrophobic				
Cory Bros HEPA Filter/HME	9.0	10.2	13.8	8.2
DAR Sterivent Mini	0.6	0.7	243	287
Pall BB100	0.7	0.8	92.4	97.5
Pall BB25	0.6	0.8	471	458
Portex Thermovent HEPA	0.5	0.6	34.7	52.8
Electrostatic: Intersurgical				
Clear-Therm	345	2020	8480	30 800
Filta-Therm	11.8	86.3	968	1990
Electrostatic: others				
Cory Bros FilterVent	14 300	19 300	103 000	110 000
DAR Hygrobac S	6330	6830	67 200	67 600
Datex-Ohmeda HMEF1000	13 600	4970	85 800	60 500
Gibeck Humid-Vent Filter Compact	16 700	18 900	103 000	147 000
Ventalink Adult	5340	6480	77 800	129 000

Values quoted to three significant figures or one decimal place. The limit of detection is ≈ 0.5 MPV for the bacterial challenge, corresponding to a filtration efficiency > 99.999995%. For some filters, the MPV was at the limit of detection. For these filters, the geometric mean was calculated using the limit of detection as the MPV value.

the unused and conditioned filters ($p = 0.47$ and 0.76 for the bacterial and viral challenges, respectively).

Making the same comparison separately for pleated hydrophobic or electrostatic filters (Table 4), gave p-values of 0.042 and 0.92 for the comparison between unused and conditioned pleated hydrophobic filters for the bacterial and viral challenges, respectively, and 0.81 and 0.47 for the comparison of unused and conditioned electrostatic filters for bacterial and viral challenges, respectively. As $p = 0.042$ arises from one of several similar tests it does not represent a truly significant result and the mean difference in *actual* MPV (conditioned – unused) was only 0.2 (statistical significance arises only because the MPVs in this group were all very small).

Table 4 Bacterial and viral microbial penetration values: geometric mean (95% confidence limits).

	Pleated hydrophobic filters			Electrostatic filters			Ratio (p-value) electrostatic/pleated hydrophobic	
Challenge	**Unused**	**Conditioned**	**Ratio (p-value) conditioned/ unused**	**Unused**	**Conditioned**	**Ratio (p-value) conditioned/ unused**	**Unused**	**Conditioned**
Bacterial MPV	1.0 (0.4, 2.3)	1.2 (0.6, 2.7)	1.2 (0.042)	2390 (728, 7860)	3908 (1720, 8890)	1.6 (0.81)	2390 (< 0.0001)	3260 (< 0.0001)
Viral MPV	87 (33, 230)	89 (35, 228)	1.0 (0.92)	32 600 (15 000, 70 800)	47 200 (24 600, 90 700)	1.5 (0.47)	374 (< 0.0001)	531 (< 0.0001)
Ratio V_{MPV}/B_{MPV} p-value	87 (< 0.0001)	74 (< 0.0001)		14 (0.0001)	12 (< 0.0001)			

The microbial penetration value (MPV) is the number of microbes passing through the breathing system filter per 10 million microbes in the challenge. p-values for differences between groups calculated using $\log_{10}$(MPV).

Table 5 Initial pressure decrease across the breathing system filters at 30 l.min^{-1}, increase in pressure after conditioning, and the percentage increase in weight of the filters after conditioning ($n = 3$ for each filter).

Breathing system filter	Mean (SD) initial pressure decrease (Pa)	Mean increase in pressure (%)	Mean (SD) increase in weight (g)
Pleated hydrophobic			
HEPA Filter/HME	119 (3)	12	5.0 (0.7)
Sterivent Mini	181 (11)	− 4	2.2 (0.9)
BB100	72 (2)	37	8.2 (3.0)
BB25	143 (8)	− 1	0.1 (0.2)
Thermovent HEPA	135 (8)	23	2.8 (1.8)
Electrostatic: intersurgical			
Clear-Therm	95 (4)	71	9.4 (0.2)
Filta-Therm	123 (5)	44	7.0 (0.4)
Electrostatic: other manufacturers			
FilterVent	78 (5)	12	4.8 (0.1)
Hygrobac 'S'	207 (3)	22	2.8 (0.1)
HMEF1000	75 (5)	8	5.6 (0.3)
Humid-Vent Filter Compact	82 (7)	121	5.9 (0.5)
Ventalink Adult	138 (8)	13	5.8 (0.4)

Differences in $\log_{10}$(MPV) values before and after use were generally small, but the penetration for the two Intersurgical devices was 2–10 times greater after conditioning than before (Fig. 2).

Conditioning increased the pressure decrease across the breathing system filters by $< 25\%$ in most filters, but more than doubled it in one (Table 5), and increased the weight of each filter by 0–9 g (Table 5).

Room temperature during the tests was in the range 21 ± 4 °C, a slightly larger variation than that specified in ISO 9360 (23 ± 2 °C).

Discussion

Testing to the draft standard for the bacterial and viral filtration performance of breathing system filters [12] demonstrated differences in the filtration performance of breathing system filters between: different filter types, bacterial and viral challenges, different examples of the same type of filter, and before and after conditioning for some filters.

The results of this study confirm those obtained previously, in which the filtration performance of pleated hydrophobic membrane filters was demonstrated to be markedly greater than that of electrostatic filters [4–9]. The exception is the Intersurgical Filta-Therm [7], again confirmed in this study. It is known that the filter medium used in the Intersurgical devices is different from those used in the other electrostatic filters.

Different test methods used in previous studies have not enabled direct interstudy comparisons to be made. However, adoption of the draft standard should allow a direct comparison between different devices allowing an informed choice to be made when comparing devices reported in different publications. In addition, information on the moisture output, pressure decrease and compressible volume of the devices, and whether the connectors are correctly dimensioned, is available from the Medical Devices Agency [21].

Microbes can pass into the breathing system from the patient in two ways, either by infected droplets carried in the gas stream or by contaminated liquid or condensate. The draft standard determines the ability of a breathing system filter to prevent only gas-borne microbes from passing from the patient to the breathing system or vice versa. The gas-borne infection route is accepted for some forms of bacterial infection, such as *Pseudomonas aeruginosa* [1], and viral infections, such as influenza [22]. However, infection resulting from liquid transfer from the breathing system to the patient is considered to be an important route.

A similar droplet size distribution was used for both the bacterial and viral challenges. The droplet size distribution ensured that each droplet produced by the three-jet Collison spray for the bacterial challenge contained, at most, one bacterium. However, for droplets containing viruses, the droplet may contain more than one virus together with cell debris and solute crystals. The MPVs for the viruses were much greater than for the bacteria. We hypothesise that, since the droplet sizes are the same, both the bacterial and viral droplets impact on the filter media, but that the viruses, released from the droplet after contact with moisture accumulated on the filter, can be driven onwards by the flow of gas, whereas the bacteria remain attached to the filter media.

The weight of all the breathing system filters tested increased after conditioning. This increase in weight was due to condensation of and absorption of water vapour within the breathing system filter. This suggests that the filter elements were exposed to sufficiently high humidity to simulate clinical use, as intended, even though dry air was delivered to the machine side of the breathing system filter. In anaesthesia, humidity present in the breathing system may increase the amount of condensation that occurs, but this will be offset by the shorter period of use. The mean pressure decrease across 10 of the 12 different breathing system filters tested also increased after conditioning.

The bacterial and viral MPVs of the two Intersurgical electrostatic filters increased following conditioning. However, even after 24 h, the MPVs for one of these devices, the Filta-Therm, were still markedly less than for other electrostatic filters. The remaining electrostatic devices, and all the pleated hydrophobic devices, were not greatly affected by conditioning.

The draft standard specifies challenging breathing system filters with aerosols. Aerosols require energy to be produced, an increase in energy reducing the droplet size. Droplet sizes below ≈ 8 μm are important, as these droplets can remain airborne for long periods, and can be deposited deep in the patient's respiratory tract. Larger droplets settle out rapidly. Large droplets are produced during speaking, while small droplets are produced during coughing and sneezing. Very few droplets are produced during normal breathing [23].

The minimum bacterial and viral challenges specified in the draft standard contain large numbers of microbes: 10^7 and 10^8 for the bacterial and viral challenge, respectively. These large numbers are necessary, in part, to cover the wide range of filtration performance exhibited by breathing system filters, demonstrated by this study. Here, the actual challenges were greater than the minimum levels specified in the standard: 2–5 times greater for the bacterial challenge, and 50–170 times for the viral challenge.

Gerone and colleagues [24] measured the number of droplets and the total volume expelled during a representative cough from a person infected with coxsackievirus A, type 21. The total number of droplets in the size range 1–8 μm was 2.45×10^4 with a total volume of 1.56×10^{-7} ml. Even in patients with high titres of viruses, the viral titre is rarely greater than 10^7 ml$^{-1.}$ It is, therefore, unlikely that one cough would produce more then a few infected droplets.

These small numbers have been confirmed by measuring the number of infected droplets expelled during a cough by an infected patient [25]. Patients with various infections were asked to cough six times. During the series of six coughs from patients infected with haemolytic streptococci, droplets containing the pathogen were found to be expelled by 39 of 87 persons with infected throats. In all, 1109 infected droplets were expelled during 522 coughs, that is, about two infected droplets for each cough. In a similar experiment on patients with faucial diphtheria, 48 infected droplets were expelled during 300 coughs, that is about one infected droplet for every six coughs; with patients with open pulmonary tuberculosis, 36 infected droplets were collected during 120 coughs. Therefore, a breathing system filter with only a modest filtration performance would remove a large majority of infected droplets expelled during a cough.

Cross-infection requires that any microbes expelled by one patient have to enter a second patient. Microbes expelled by a patient which do pass through a breathing system filter will attach to the walls of the breathing system. Ibrahim and Perceval [26] demonstrated that air passing through breathing system tubing 'seeded' with microbes did not become contaminated. Nielsen and colleagues measured a mean bacterial count of 0.385 l^{-1} (range 0.033–1.298 l^{-1}) in a 50% oxygen in nitrous oxide mixture delivered through a 'seeded' breathing system [27]. Therefore, given the small number of microbes likely to be expelled by patients, and the small amount of contamination of the gas as it passes through the breathing system, it is unlikely that cross-infection will occur when any of the breathing system filters tested in this study are used, as far as gas-borne cross-infection is concerned. It has been demonstrated that pleated hydrophobic filters prevent liquid contamination of breathing systems, whereas the ability of electrostatic filters to prevent liquid contamination depends on the volume of liquid, the orientation of the filter membrane, and the internal volume of the filter housing [28].

This study has demonstrated that pleated hydrophobic membrane filters have a superior filtration performance compared with electrostatic filters, and that there is a difference between the filtration performance of electrostatic filters from different manufacturers. However, the necessity of choosing a breathing system filter with a high filtration performance remains controversial.

Acknowledgments

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