

## ORIGINAL ARTICLE

## Evaluation of a new device against bacterial penetration

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**Objective:** This study analyzed how effectively bacteria penetrate through a new filtering device (Muski™) aimed at providing temporary protection against hazardous air contaminants. **Materials and methods:** A  $1.5\text{--}7 \times 10^8$  cfu/ml bacterial or spore suspension was aerosolized. Fragments of the Muski filter, previously sterilized by gamma rays, were placed in a sterile stainless steel Seitz filter holder, plugged into an Erlenmeyer vacuum flask connected to a vacuum pump to force the bacteria aerosol through the Muski filter. As a control the same apparatus was used without the Muski filter. To evaluate the bacteria passing through the apparatus, diluent solution was introduced in the Erlenmeyer flask, which was then subjected to mechanical shaking. Both the contents of Erlenmeyer flask and the diluent solution, used to rinse out the Erlenmeyer flask, were filtered through a membrane filtration apparatus. The filtration membrane was transferred to the surface of a Petri dish containing a suitable culture medium for the species of bacteria tested. After incubation the number of developed cfu (colony forming units) was evaluated. **Results:** The presence of the Muski filter in the experimental aspiration apparatus strongly reduced the passage of bacteria and spores of varying shapes and sizes that were experimentally dispersed into the air. The logarithmic reduction varied from 1.77 to 3.90 for the vegetative forms and from 1.24 to 2.05 for bacillus spores. **Conclusion:** The results demonstrate that the Muski device is a valid alternative to traditional respirators, including those of the last generation. Nevertheless, the Muski device must be considered as a 'first aid' device, to be carried by the person to permit a safe, rapid escape from the area presumed to be contaminated by bacteria.

**Key words:** face mask, infection control, bioterrorism**Introduction**

Recent terrorist attacks, even in countries that were once considered to be immune, have brought the distressing phenomenon of bioterrorist strike to the fore. These events may release dangerous micro-organisms such as the causative agent of anthrax. Although such attacks have not yet occurred, they are possible and imply a real danger that should not be underestimated. Breathing apparatus to protect against dusts and gases, used in industry, in hospital environments and in preventative systems, have now become essential for the protection of individuals in cases of biological terror as well as in contaminated environments. Among the type N respirators, N95 is the most commonly used and is able to block 96% of the smallest particles (0.1–0.3  $\mu\text{m}$ ). It has practically replaced the less efficient DM (Dust Mist) and DMF (Dust Mist Fume) respirators, able to block 72% and 82% of the particles, respectively (1). The masks used in surgical practice were originally conceived to protect the patient from infective

agents that could be transmitted from the operators through coughing or sneezing or produced or through simple speech or to protect the personnel from contamination from the spray used in dental treatments (2). The droplets produced in these cases are  $\geq 4 \mu\text{m}$  in diameter (3) and are effectively blocked by surgical masks, although particles  $\leq 1 \mu\text{m}$  can easily pass through such masks (4,5). Half face masks protecting nose and mouth only, such as P2 (European) or N95 (US), or bulky masks specifically designed for CBR use, are expensive and unsuitable as protection in situations where a disposable immediately accessible, compact, handy, and portable device is required.

For both respirators and surgical masks, bacterial penetration is in inverse proportion to their size. Consequently, rod-shaped bacteria seem less able to penetrate than spherical bacteria (6–8). However, despite the demonstrated effectiveness of N95 respirators vs surgical masks in protecting against sudden bioterrorist attack, they are quite expensive and their size make them difficult to apply for the

0 general population. In emergency situations, the  
 'man-on-the-street' would not be protected and  
 would, therefore, be exposed to chemical, biochemical  
 and/or biological contamination. The use of a  
 5 small, simple device that is cheap and easy to wear  
 would provide temporary protection from potentially  
 hazardous atmospheric contaminants until the  
 wearer is able to leave the area and reach a safe,  
 protected zone. In addition, a device like Muski  
 10 might also be used by operators exposed to the  
 aerosol contamination from infected patients, as for  
 example, patients affected by pulmonary tuberculosis  
 (TB), a disease which today presents a new  
 outbreak in developed countries because of a high  
 level of immigration from countries of the third  
 15 world. The present study tested the effectiveness  
 against bacterial penetration of a new device  
 (Muski<sup>TM</sup>; Starburst Technologies Ltd, NJ, USA;  
 www.muski.com) which appears able to meet the  
 above requirements.

## Materials and methods

### *Technical characteristics of the device*

25 Muski is a bag-shaped device that can easily be  
 slipped on over the wearer's head and closed tight  
 around the neck with a rubber band, protecting the  
 face and eyes from the toxic effects of chemical or  
 biological agents (Figure 1). The air only enters



Figure 1. Muski in use: a man wearing a Muski filter.

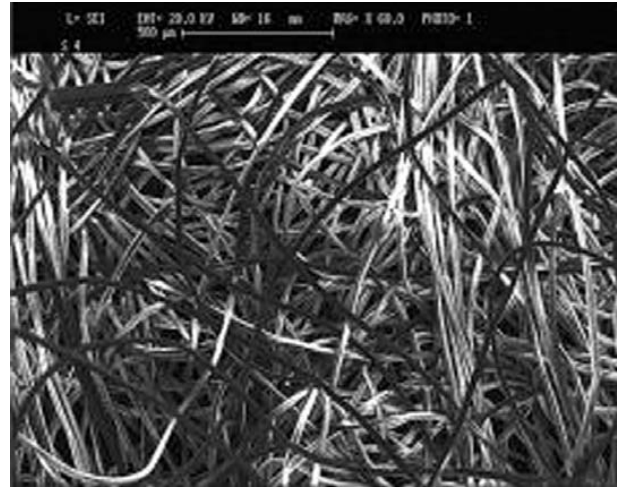


Figure 2. Outer layer of the Muski filter ( $\times 60$ ).

through the filters set in the mask. A one-way  
 precision valve enables the person to exhale, making  
 escape from the contaminated area safer and more  
 comfortable. The presence of a single-layer, activated  
 carbon filter also prevents inhalation of fumes  
 and toxic gases. This device is also small enough to  
 be easily carried inside a pocket or handbag.

The filter of the Muski device has the following  
 characteristics. Layer 1: an outer layer of medical-  
 grade, non-woven fabric that acts as a protective  
 barrier for the inner layers. It can filter substances  
 larger than  $2 \mu\text{m}$  (Figure 2). Layer 2: an activated  
 carbon layer,  $200 \text{ g/m}^2$  of which  $114 \text{ g/m}^2$  are  
 charcoal and  $86 \text{ g/m}^2$  polyester fibres. This layer  
 filters smoke, odors, etc. Dust absorption capacity is  
 $14.6 \text{ g}/16 \text{ g}$  (91.25%) at an air flow rate of  $0.283 \text{ m}^3$   
 (Figure 3). Layer 3: a layer made of 100% poly-  
 propylene non-woven medium with an outer layer of  
 spunbonded fibres for strength and inner layer of  
 meltblown. This layer filters substances with a  
 particle size of  $0.3 \mu\text{m}$  or more. Air permeability is  
 $1.556 \text{ m}^3/\text{min}/0.0929 \text{ m}^2$ ; filtering capacity for

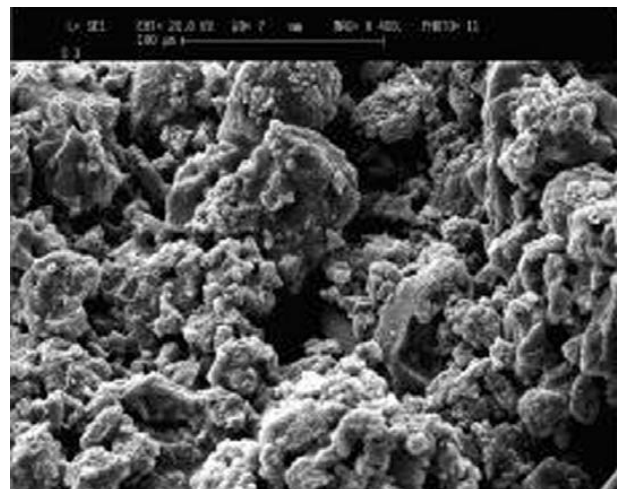


Figure 3. Carbon layer of the Muski filter ( $\times 400$ ).

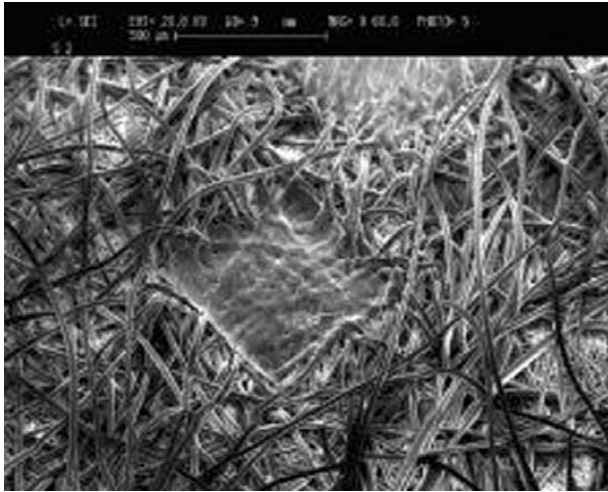


Figure 4. Intermediate layer of Muski filter ( $\times 60$ ).

particles of different size is 0.3–0.5  $\mu\text{m}$  38.6%, 0.5–0.7  $\mu\text{m}$  68.8%, 0.7–1.0  $\mu\text{m}$  78.6%, 1.0–2.0  $\mu\text{m}$  83.7% (Figure 4). Layer 4: an inner layer with the same characteristics as the outer layer (Figure 5).

Layers 1 and 4 also have antiseptic properties as they include 0.3% of a heteroaromatic ammonium salt, cetylpyridinium halide (CPC).

Two different types of Muski filter were evaluated: Muski filters A and B. Type A layer 3 is slightly thinner than the same material in type B. The difference is in the compression of the material and the permeability functions, resulting in easier, more comfortable respiration.

#### Evaluation of the efficiency of the Muski filter as a barrier against bacterial penetration

Different species of bacteria – non-pathogenic or poorly pathogenic when airborne – were selected for their different dimensions or shapes (spherical or rod-shaped) similar to those of the bacteria that could be used in bioterrorism: *Yersinia pestis* or spores of *Bacillus anthracis* (Table I). They present

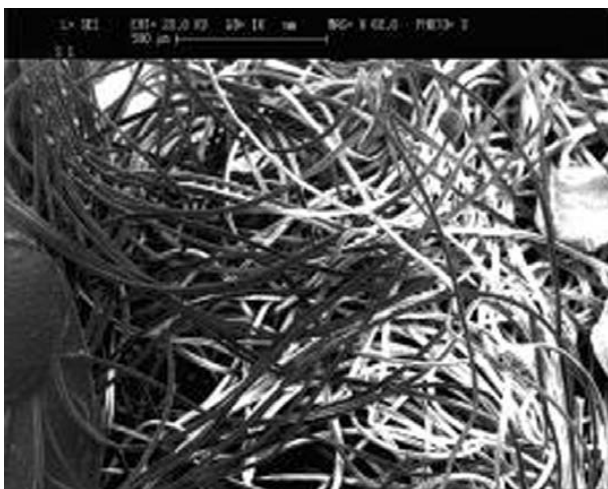


Figure 5. Inner layer of Muski filter ( $\times 60$ ).

Table I. Dimensions (average and SD) of two bacterial species that could be used in terrorist attacks.\*

Type of bacteria	Dimensions in $\mu\text{m}$	Average aspect ratio†
<i>Yersinia pestis</i>	1.50 $\times$ 0.70	2.1
<i>Bacillus anthracis</i> spores	2.50 $\times$ 1.12	2.2

\*Dimensions given as diameter for spherical bacteria, length and width for spores and rod-shaped bacteria. †The average aspect ratio is calculated by dividing the average length by average diameter of the bacterium or spore.

theoretically different abilities to pass through the Muski filter.

A  $1.5\text{--}7 \times 10^8$  cfu/ml bacterial or spore suspension in a 0.1% tryptone solution of each tested bacterium – *Streptococcus pyogenes* ATCC 19615, *Staphylococcus cohnii* ATCC 35662, *Serratia marcescens* ATCC 8100, *Pseudomonas fluorescens* 49838, *Bacillus clausii* spores (Sonafi-Syntelabo Oto S.p.a., Milan, Italy), *Bacillus stearothermophilus* spores ATCC 10149, *Bacillus subtilis* spores ATCC 6633 – was washed three times to remove residual surface material such as bacterial slime (which is responsible for bacterial clusters) and aerosolized using a medical device for aerosol therapy. Fragments of the Muski filter, previously sterilized by gamma rays, were placed in a stainless steel Seitz filter holder, sterilized by steam and plugged into an Erlenmeyer vacuum flask. Then, using a sterile tube (the ends made of Teflon, the center of glass containing a cotton filter) this was connected to a vacuum pump to force the bacterial aerosol through the Muski filter. As a control the same apparatus was used without the Muski filter. The aerosol generator and Seitz filter were set approximately 25 cm apart. After 10 minutes, aerosolization and vacuum aspirations (0.4  $\text{m}^3$ ) were stopped. To evaluate the bacteria passing through the apparatus, 50 ml of a diluent solution (tryptone sodium chloride) was introduced in the Erlenmeyer flask, which was then subjected to mechanical shaking. Both the contents of Erlenmeyer flask and the diluent solution (50 ml), used twice to rinse out the Erlenmeyer flask, were filtered through a membrane filtration apparatus. The filtration membrane was transferred to the surface of a Petri dish containing a suitable culture medium for the species of bacteria tested. The following culture media were used. (i) *Pseudomonas* isolation agar (Difco) for *Pseudomonas fluorescens*. (ii) Mannitol salt agar (Biomerieux) for *Staphylococcus cohnii*. (iii) Streptococcus selective medium (Oxoid) with 5% defibrinated horse blood for *Streptococcus pyogenes*. (iv) Hektoen enteric agar (Biomerieux) for *Serratia marcescens*. (v) Tryptone soya agar (Oxoid) for *Bacillus subtilis*, *Bacillus stearothermophilus* and *Bacillus clausii*.

All Petri dishes were incubated at  $36 \pm 1^\circ\text{C}$  for 24–48 hours and the number of developed cfu was

Table II. Dimensions (average and SD) of the different species of bacteria tested.\*

Type of bacteria	Dimensions in $\mu\text{m}$	Average aspect ratio†
<i>Staphylococcus cohnii</i>	$0.70 \pm 0.08$	1.0
<i>Streptococcus pyogenes</i>	$0.80 \pm 0.16$	1.0
<i>Serratia marcescens</i>	$1.10 \pm 0.60 \times 0.37 \pm 0.04$	3.0
<i>Pseudomonas fluorescens</i>	$1.50 \pm 0.49 \times 0.51 \pm 0.03$	2.9
<i>Bacillus stearothermophilus</i> spores	$0.86 \pm 0.13 \times 0.53 \pm 0.04$	1.6
<i>Bacillus clausii</i> spores	$1.01 \pm 0.13 \times 0.57 \pm 0.05$	1.8
<i>Bacillus subtilis</i> spores	$1.25 \pm 0.15 \times 0.58 \pm 0.07$	2.2

\*Dimensions given as diameter for spherical bacteria, length and width for spores and rod-shaped bacteria. †The average aspect ratio is calculated by dividing the average length by average diameter of the bacterium or spore.

evaluated. For *Bacillus stearothermophilus* the incubation temperature was  $56 \pm 1^\circ\text{C}$ . To detect slow-growing bacteria, the culture time was extended to a week. No significant differences in the numbers of cfu were observed between the 24 and 48 hour cultures and the 1 week cultures. In all tests, the API system (bioMérieux) was used to identify the developed cfu. The developed bacteria were always the same as were originally aerosolized.

To evaluate the possible correlation between the size of the different tested bacteria and spores and their ability to cross through the filter, droplets of bacteria or spore suspensions were spread over the surface of a cover glass. The different samples were all fixed for 3 hours at  $4^\circ\text{C}$  in a 3% glutaraldehyde solution in a 0.1 M phosphate buffer at pH 7.4. After several washings in phosphate buffer, the material was dehydrated by passing it through

solutions with increasing concentrations of ethyl alcohol, through a propylene oxide solution, and finally subjecting it to critical point drying. The samples were then sputter-coated with gold palladium (Edwards sputter-coater S150) and examined under a scanning electron microscope (Cambridge Stereoscan S360). The dimensions of 50 bacteria cells or spores of each species were then measured under SEM and the mean dimensions and standard deviation (SD) were calculated for each tested bacterial species (Table II).

## Results

The results are summarized in Tables III and IV.

In our experimental air aspiration, the presence of the Muski filter significantly reduced the passage of

Table III. Number of cfu developed by the microorganisms that passed through the experimental aspiration device with or without Muski filter, logarithmic and percentage reduction with the Muski filter.

Test microorganism	No. of cfu	Type of Muski filter	Log value	Log reduction factor with Muski filter	
<i>Staphylococcus cohnii</i>	With Muski	0	A	0.00	2.71
	Without Muski	516		2.71	
	With Muski	0	B	0.00	3.90
	Without Muski	8000		3.90	
	With Muski	15	A	1.18	2.62
	Without Muski	6300		3.80	
<i>Streptococcus pyogenes</i>	With Muski	0	B	0.00	2.37
	Without Muski	233		2.37	
	With Muski	0	B	0.00	2.92
	Without Muski	840		2.92	
	With Muski	2	A	0.30	2.92
	Without Muski	1650		3.22	
<i>Serratia marcescens</i>	With Muski	5	A	0.70	3.20
	Without Muski	8004		3.90	
	With Muski	0	B	0.00	2.48
	Without Muski	304		1.48	
	With Muski	2	A	0.30	3.30
	Without Muski	4000		3.60	
<i>Pseudomonas fluorescens</i>	With Muski	3	A	0.48	1.77
	Without Muski	176		2.25	
	With Muski	9	B	0.95	1.78
	Without Muski	538		2.73	
	With Muski	3	A	0.48	2.95
	Without Muski	850		2.93	

cfu, colony-forming units.

Table IV. Number of cfu developed by the microorganisms that passed through the experimental aspiration device with or without Muski filter, logarithmic and percentage reduction with the Muski filter.

Test microorganism	No. of cfu	Type of Muski filter	Log value	Log reduction factor with Muski filter	
Spore <i>Bacillus clausii</i>	With Muski	95	A	1.98	1.67
	Without Muski	4440		3.65	
	With Muski	95	B	1.98	1.62
	Without Muski	4025		3.60	
	With Muski	17	B	1.23	1.68
	Without Muski	680		2.83	
Spore <i>Bacillus stearothersophilus</i>	With Muski	46	B	1.66	1.40
	Without Muski	1148		3.06	
	With Muski	3	A	0.48	2.05
	Without Muski	340		2.53	
	With Muski	9	B	0.95	1.66
	Without Muski	411		2.61	
Spore <i>Bacillus subtilis</i>	With Muski	30	B	1.48	1.24
	Without Muski	523		2.72	
	With Muski	4	B	0.60	1.60
	Without Muski	157		2.20	
	With Muski	18	A	1.26	1.44
	Without Muski	496		2.70	

cfu, colony-forming units.

aerosolized bacteria and spores. In fact, in the case of aerosol spraying of vegetative forms of bacteria, the logarithmic reduction varied from 1.77 for *Pseudomonas fluorescens* to 3.90 for *Staphylococcus cohnii*, while for aerosolized spores of different *Bacillus* species it ranged between 1.24 for *Bacillus subtilis* and 2.05 for *Bacillus stearothersophilus*. There does not appear to be any correlation between the size of the bacteria or spores and their ability to pass through the filters comprising the Muski unit. Moreover, there does not appear to be any evident difference between the ability of spherical bacteria (*Staphylococcus cohnii* and *Streptococcus pyogenes*) and rod-shaped bacteria (*Serratia marcescens* and *Pseudomonas fluorescens*) to pass through the Muski filter. After running tests using the filter as a whole – i.e. layers 1, 2, 3 and 4 together – we then separately tested the blocking action of the individual layers of the filter. To evaluate the ability of bacteria to penetrate the various filtering layers, we chose *Staphylococcus cohnii* as the test organism because it is the smallest of the bacteria used in the study. However, because of the adhesion between the outermost layer of micro-fibre and the active carbon

layer, it proved impossible to separate the two. Therefore layers 1 (micro-fibre) and 2 (activated carbon) were tested together and the results refer to their combined action. The combination of layers 1+2 provided the highest logarithmic reduction in bacterial penetration (2.26). This was followed by layer 3, which is structurally able to filter particles  $\geq 0.3 \mu\text{m}$  (1.71). Layer 4, made up of the micro-fibre able to block particles of  $\geq 2 \mu\text{m}$ , showed a logarithmic reduction of 0.48. Layer 2, made up of the activated carbon presented a logarithmic reduction of 0.99.

If we take the results obtained with the activated carbon layer 2 alone (a logarithmic reduction of 0.99) and compare them with the combined layers 1+2 (a logarithmic reduction of 2.26), we can see that the reduction is markedly lower. However, it is worth noting that the porosity of layer 2, able to filter fumes and gases, is certainly able to block bacteria. As layers 1 and 2 were separated mechanically, we cannot rule out the possibility that removal of layer 1 could have damaged the activated carbon layer (Table V). It is quite difficult to compare the sensitivity of the various bacteria tested vs the

Table V. Number of cfu developed in the presence or absence of the various layers making up the type A filter, logarithmic and percentage reduction with various layers making up the type A filter.

Organism	No. of cfu	Layers	Log value	Log reduction factor	
<i>Staphylococcus cohnii</i>	With Muski	130	Layers 1+2	2.11	2.66
	Without Muski	23200		4.37	
	With Muski	103	Layer 3	2.01	1.71
	Without Muski	5250		3.72	
	With Muski	4800	Layer 4	3.68	0.48
	Without Muski	14400		4.16	
	With Muski	90	Layer 2	1.95	0.99
	Without Muski	871		2.94	

Table VI. Comparison between the technical peculiarities and the protective efficiency of Muski and of N95 or P2.

Parameter	Standard N95 (P2)	Muski™
Filtration of particles	95% (94%)	Tested for: filtration of 99% of particles >0.1 µm
Filtration of microorganisms	Not tested	Tested for: filtration of 99.9% of microorganisms
Range of protection assuming that avian flu will spread from avian hosts to humans*	From contamination by aspiration and digestion	Besides the respiratory orifices the hood protects eyes, ears, bare skin of head and neck†
Fitting	Half face masks do not fit all face types, resulting in potential leakage of contaminated air Verbal communication will induce leakage	Hood accommodates all types of head shapes, including special features like moustaches or beards and completely seals the space inside the hood so that all the inhaled air enters only through the filter Verbal communication is possible, including use of telephone
Protecting the eyes	Half face masks do not protect the eyes	Hood protects the eyes and also allows wearing of glasses if needed
Economical concept in the relevant context	'Level A' protection would be optimal but its high cost would dictate multiple use, leading to potential cross-infection	Minimal but adequate protection at relatively low cost allows adoption of the concept of using a disposable device, thereby isolating contaminants
Duration of use	'Level A': about 30 minutes	Not limited by time of use, can be used until clogged
Ability to drink water during work (essential if workers are wearing isolated garments)	Half face mask does not permit drinking of water while in contaminated areas	Hood type masks permit safe drinking via a tube connected to a filtered, protected water bottle
Disposal	Eventually contaminated mask needs special disposal solution	Used hood should be removed by turning it inside-out, to accommodate the used gloves and garments and to be sealed by the rubber bands, thereby not presenting immediate hazard regarding disposal

\*There is no precise description yet as to the way avian influenza virus is transmitted from bird to bird or from birds to humans. †In any case complementary protection will be necessary such as garments, gloves and boots.

disinfectant (cetylpyridinium halide) with their ability to pass through the various layers of the filter alive. We must also consider that the reduction in the bacteria passing through the filter of the device being tested is determined both by the structure of the various layers acting as physical filter, and by the presence of the disinfectant in the inner-most and outer-most layers, which can also have a biocidal effect. In fact, using our experimental protocol to evaluate the reduction in the number of bacteria, we only consider the bacteria able to form colonies; that is those which actually passed through the various layers of the filter unharmed.

## Discussion

From the results obtained it is possible to assert that, as a whole, the various layers comprising the filter in the mask provide a valid obstacle to the passage of bacteria and spores. In fact, they proved able to achieve a logarithmic reduction of between 1.77 and 3.90 in the living bacteria. On the other hand, the logarithmic percentage of spores blocked by the filters was slightly lower, varying between 1.24 and 2.05. As a whole, it must be pointed out that there did not appear to be any significant difference between the two types of masks, A and B (the filters were thinner in type A masks). Moreover, in contrast with what has been found by other authors (6,7) no significant difference was

found in the ability of the smaller, spherical bacteria to cross through the filter vs the larger, rod-shaped bacteria.

No direct correspondence could be observed between the size of the bacteria and their ability to pass through the filters in the mask. Cocci and *Serratia* showed a greater reduction than did *Pseudomonas* and the spores, which are on the average smaller. This phenomenon is difficult to explain. However, other factors besides size may affect passage through the filter, such as the presence of residues of the culture medium or slime which are stuck to the bacteria and are difficult to remove completely by bacterial suspension wash processes. Moreover, this could accidentally facilitate adhesion of a certain number of bacteria.

*Bacillus* spores were better able to pass through the various layers of the filter unharmed than the other bacteria tested. The vegetative forms and spores used in the present study were not pathogenic or only slightly pathogenic at the concentrations used. They were chosen because their size and shape reflected those of highly pathogenic bacteria that could potentially be used for bioterrorist attacks. In the light of current day threats of contaminating diseases (such as avian flu) and contagious diseases (such as TB – which releases infectious bacteria), there is a gap in the market for an effective, disposable, inexpensive and compact protective hood mask. The hood mask should be available

0 and accessible as emergency first aid, to all levels of  
 the population, for easy, quick and safe donning in  
 life-threatening situations. Because of their small  
 size, testing against viruses is difficult. Viruses  
 usually attach themselves to human tissue or enter  
 5 cells, thus creating particles in sizes that exceed  
 0.3  $\mu\text{m}$ . These cells are filtered by Muski™ filters,  
 thus preventing passage of the virus attached to the  
 cells via the filters (Table VI).

10 As a whole, our results show that, in terms of  
 effectiveness, the Muski device is a valid alternative  
 to traditional respirators, even those of the latest  
 generation (1). Moreover a great advantage of this  
 device is its small size, the ease with which it can be  
 worn and removed, and last but not least, its low  
 15 price. Indeed, in the case of a bioterrorist attack, the  
 entire population could be protected, with no  
 exceptions. Therefore, protection devices must be  
 cheap enough to be accessible to the individual, and  
 should not prove an excessive financial burden for  
 the community as a whole. Nevertheless, the Muski  
 device must be considered as a 'first aid' device, to  
 be carried by the person to permit a safe, rapid  
 escape from the area presumed to be contaminated  
 by bacteria. Prolonged periods, working in such  
 25 contaminated areas, require more sophisticated  
 filtering and protective devices that are not

60 exclusively limited to the head, but extended to the  
 entire body surface.

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