

BACTERIAL SURVIVAL ON RESPIRATOR FILTERS AND SURGICAL MASKS

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ABSTRACT

The Centers for Disease Control and Prevention recently developed recommendations for respirators worn to minimize exposures to *Mycobacterium tuberculosis*. These recommendations include criteria for filters and facial fit, but do not address how respirators are worn or disposed of. Handling may increase the risk of infection if loaded organisms remain viable.

Respirator filters were challenged with aerosols containing three bacteria: *Mycobacterium abscessus*, *Staphylococcus epidermidis*, and *Bacillus subtilis* subsp. *niger*. Elution and surface contact sampling were used to recover organisms from filters after loading and following 5 days storage at 85% RH.

Almost all filters showed culturable organisms immediately after loading. After storage, filters showed the least culturability for *M. abscessus* (20% of all filters) followed by *S. epidermidis* (61%), while *B. subtilis* remained highly culturable (98%). A similar trend was seen for the number of organisms recovered before and after storage (*M. abscessus* demonstrated the least survival and *B. subtilis* the most).

INTRODUCTION

Current certification and use policies for respirators in environments containing non-biological aerosols do not address exposure to contaminants once captured by filters, because there is no evidence that they pose a further risk to the respirator user. However, this may not be true when the contaminant includes viable cells. The potential for captured pathogens to survive and replicate on filter media must be considered.

This research evaluated the long-term culturability of three species of bacteria following capture on respirator and surgical mask filter media. Culturable bacteria were recovered from filters at two separate times: 1) immediately following loading and 2) after five days of storage at 85% relative humidity. Two assessment methods were employed. In the first, bacteria were eluted from throughout the filter; the second involved agar-contact surface sampling of both the front and back of the filter. Organism release from filters was not evaluated in these experiments.

Surgical masks have traditionally been used in the health care setting to protect the patient from infectious particles expelled by the worker (Davis, 1991). However, these masks, which do not fit tightly to the face, will not protect the wearer from inhaling airborne particles. The growing interest in the protection of health care workers from infectious aerosols has resulted in the use of respirators in medical settings. Further impetus comes from the Centers for Disease

Control and Prevention (CDC) and the Occupational Safety and Health Administration (OSHA), both of which recommend that National Institute for Occupational Safety and Health (NIOSH)-approved respirators be used to minimize exposures to *Mycobacterium tuberculosis* (CDC, 1994; Clark, 1993). To control costs in these facilities disposable respirators are sometimes reused numerous times until the fit is compromised (Nettleman et al., 1994; Rivera et al., 1997; Adal et al., 1994; Jackson, 1994; Clark, 1993). The consequences of storage conditions and respirator reuse have not been evaluated and the risk of exposure to organisms released from filter media has not been thoroughly addressed by CDC or OSHA.

One investigator found that storage of respirator filters in high humidity conditions resulted in heavy microbial contamination, particularly when the filter material was biodegradable by microorganisms (Pasanen et al., 1993). Two filter types were loaded in an agricultural barn and in a wastewater treatment plant and the culturable organism load was evaluated immediately following loading and after storage at 98% relative humidity (RH) for up to 35 days. Following incubation the actinomycete and other bacterial and spore concentrations were one to three orders of magnitude higher in both filters; the fungal spore concentration increased in one filter but decreased in the other. Another study investigated growth of a fungus (*Stachybotrys atra corda*) on respirator filters and found strong evidence of visible growth on filters consisting of 85% cellulose under conditions of 100% RH (Pasanen et al., 1994).

Infection from exposure to particles released from respirator filters has not been investigated, but the risk has been documented for surgical masks and other equipment used on infectious patients (Castle, 1980; JAMA, 1937). In one instance, anesthetic face masks worn by patients with active pulmonary tuberculosis were found to contain viable *M. tuberculosis* bacilli, which were shown to be infective in guinea pigs (Livingstone, 1941).

Before captured organisms can cause infection it must be assumed that there is detachment from the filter and continuing culturability. Particle detachment can occur if the adhesion forces (e.g., van der Waals, electrostatic and surface tension forces) are disrupted. This may occur due to changes in air conditions, changes in the particle, or physical agitation of the filter, resulting in the reintroduction of the particle into the airstream (Brown, 1993).

Once released from the filter, cells must remain viable to cause infection. Environmental conditions such as temperature, relative humidity and nutrient availability may either increase or decrease organism viability. With the proper environmental conditions (i.e., moisture, nutrients), viable cells may replicate and create a new reservoir. Filters are typically considered nutrient-free environments, which may prevent replication but will not lessen microbial viability. Fungi, actinomycetes, and other bacteria can utilize components of the filter media (such as cellulose) or dust and organic material captured by the filter as nutrients (Pasanen et al., 1993; Gravesen, 1979). Ventilation system filters have been suggested as potential bioaerosol sources, particularly for fungal spores (ACGIH, 1989; Darlow, 1966; Macher et al., 1995) and microbial amplification on filters has been documented in several cases (Morey, 1980).

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This study evaluated the culturability of organisms after capture on filters. Experiments were designed to determine if bacteria could survive on filter media for a period of five days when stored at 85% RH. These conditions were selected to simulate a respirator sealed in a bag immediately following use or stored in a high humidity area such as a locker room. Since lack of moisture may result in desiccation, which can cause significant losses in bacterial viability (Cox, 1995), high relative humidity was chosen to represent a supportive storage condition.

Bacteria were recovered from filter media immediately following loading and after five days of storage using two different methods (McCullough et al., (1997a)). Both qualitative and quantitative endpoints were used to evaluate differences among filters, methods and loading conditions. The presence of viable organisms on the filter was evaluated qualitatively. The presence of even one colony forming unit (CFU) following storage indicated that there was the possibility that a cell remained viable throughout storage and therefore could potentially lead to infection. The likelihood that organisms would remain viable throughout storage was estimated by a quantitative measure of "survival," which compared the CFUs recovered from the filter before and after storage.

METHODS

Organisms were loaded onto respirator filter media during efficiency tests which are described briefly here and in detail elsewhere (Brosseau et al., 1997; McCullough et al., (1997a); McCullough et al., (1997b)). Five types of surgical masks and eighteen types of respirator filters were challenged with three aerosolized bacteria. Filters from both disposable and reusable respirators were tested. Respirator filters were from the three NIOSH-approval categories: 1) dust/mist (DM), the least protective filters, 2) dust/fume/mist (DFM) and 3) high efficiency particulate air (HEPA), the most protective filters (Respiratory Protective Devices, 30 CFR Part 11, 1972). Surgical masks are not certified by NIOSH and were considered "not-approved" (NA) for this study.

Three bacteria (*Mycobacterium abscessus*, *Staphylococcus epidermidis* and *Bacillus subtilis* subsp. *Niger* (in spore form)) (described in Table 1) were utilized in these tests. *M. abscessus* and *S. epidermidis* were removed from agar plates and the *B. subtilis* were used as dried spores. All were added directly to filtered, deionized water and placed in a Collison nebulizer (BGI,) operated at 3 psi (McCullough et al., (1997a)). Bacteria were aerosolized to a target concentration of 1 particle /cm³ for NA, DM, and DFM filters and 2 particles/cm³ for HEPA filters. The aerosolization method was refined so that clumps were minimized; mean aerodynamic diameter indicated the majority of particles were single cells.

Organisms were loaded on respirator filters during aerosol challenge tests at two flows (45 and 85 L/min) and two conditions of relative humidity (30 and 70% RH). Three replicates were conducted on each filter for each organism at each test condition. Testing took an average of 30 minutes for the NA, DM and DFM filters and an average of 50 minutes for the HEPA filters. Greater loading occurred at the higher flow and the higher aerosol concentration. Tests were not

controlled for the number of bacteria loaded on a filter. Rather, the primary purpose of the aerosol challenge tests was to measure filter efficiency.

The efficiency of each filter was determined by measuring the concentration of particles and viable bacteria upstream and downstream of the filter. Those bacteria which did not penetrate the filter were collected by the filter media and their culturability and survival were evaluated during this research.

Two methods, elution and agar-contact surface sampling, were developed for recovering organisms from filters (McCullough et al., [1997a]). Immediately following loading the filter was cut in half, if necessary. In some cases, two separate filter cartridges were tested simultaneously. One cartridge or filter-half was put immediately into storage while the other was kept for sampling. A 6.45 cm² piece was cut from the center of a filter-half; a similar-sized square was cut from one quadrant of a paired filter. Elution, which involves the removal of particles attached to a substrate by transfer to a liquid, was accomplished by mixing the piece with 10 or 20 mL of a 0.05% Tween 80 solution (FischerBiotech, Pittsburgh, PA) in a 50 mL polypropylene centrifuge tube (Corning, Inc., Corning, NY). The tube was then manually shaken 200 times, after which 100 µL of liquid was plated on appropriate agar (described in Table 1). Plates were incubated and counted; results were reported as CFUs / 6.45 cm² (1 in²) of filter.

Surface sampling was accomplished with the use of replicate organism detection and counting (RODAC) plates (Becton and Dickinson, Lincoln Park, NJ) which contain a 25.8 cm² (4 in²) raised agar meniscus that contacts surfaces without interference from the rim of the plate (Favero et al, 1968). Plates were used to collect organisms from the remaining half of the filter surface. (The side of the filter which is toward the ambient air will be referred to as the front of the filter and the sampling location as "RODAC-front"; conversely, the side of the filter which is toward the wearer's face will be referred to as the back of the filter and the sampling location as "RODAC-back".) Following sampling, plates were incubated and enumerated; results were reported as CFUs / 6.45 cm² (1 in²) of filter. For RODAC plates which were overloaded a maximum value of 5000 CFUs was assigned.

The organism recovery data were evaluated with respect to two variables: "culturability" and "survival." To qualitatively evaluate the presence of viable organisms on the filter ("culturability") each sample was first assigned a categorical variable based upon the recovery of viable organisms (i.e. 1 = viable organisms present, 2 = no viable organisms present). The recovery of any viable organisms resulted in the assignment of "1", regardless of the number of organisms present. A total of 4551 samples were collected. Culturability before (C_b) and after (C_a) the 5-day storage period was determined as

$$C (a \text{ or } b) = (F_v / F_{total}) 100 \quad (1)$$

where F_v = number of samples with viable organisms and F_{total} = total number of samples. Additionally, "culturability" after storage (C_a) was statistically evaluated using categorical analysis of variance (ANOVA); predictor variables included in the statistical model are shown in Table 2. Multiple comparison tests were used to detect differences between levels of significant predictor variables ($\alpha \leq 0.05$) (Kleinbaum et al., 1988).

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TABLE 1
Organism Characteristics

	<i>Mycobacterium abscessus</i> ^a	<i>Staphylococcus epidermidis</i> ^b	<i>Bacillus subtilis</i> subsp. <i>Niger</i> ^c
Source	CDC ^d	ATCC: 14990	Bioferm Co. (San Diego, CA) ^e
Size, μm	length 1 - 4 width 0.3 - 0.5	diameter 0.5 - 1.5	length 2 - 3 width 0.5 - 0.8
Stain	acid-fast	Gram-positive	Gram-positive
Biological State	vegetative	vegetative	spore
Resistance to Desiccation ^f	medium	low	high
Agar Used in Recovery Tests	Middlebrook-7H10 (Difco, Detroit, MI)	Staphylococcus Medium 110 (Difco, Detroit, MI)	Standard Methods (Difco, Detroit, MI)
Incubation Temperature, C	36	36	21 - 24
Incubation Time, days	4 - 5	2 - 3	2 - 3

^aRunyon et al., 1974; ^bBaird-Parker, 1974; ^cGibson and Gordon, 1974; ^dChen et al., 1994; ^eJohnson et al., 1994; ^fFavero and Bond, 1991

The second endpoint, “survival” (S), was determined for the 1119 samples in which viable organisms were detected following storage. “Survival” is the ratio of CFUs measured before and after storage:

$$S = (R_a/R_b)100 \quad (2)$$

where R_a = CFUs recovered after storage and R_b = CFUs recovered before storage. For those filters for which R_a was greater than zero but R_b was zero, a value of 1 CFU was assigned for R_b . Logarithmic transformation was used to normalize the data. “Survival” was evaluated using ANOVA with the same initial model and statistical procedures as for C_a (shown in Table 2).

TABLE 2
Factors Included in the Analysis of Variance

Factors	Levels
Organism	<i>M. abscessus</i> , <i>S. epidermidis</i> , <i>B. subtilis</i>
Loading Flow, L/min	45, 85
Loading Relative Humidity, %	30, 70
Filter Approval	Non-Approved, Dust/Mist, Dust/Mist/Fume, High Efficiency
Recovery Method	Elution, RODAC-front, RODAC-back
Filter Model	23 filter models ^a

^aIndividual manufacturers and models were nested within filter approval; the specific models tested are listed in Brosseau et al., 1997.

RESULTS AND DISCUSSION

For pre-storage conditions, culturability (percent of filters with culturable organisms) ranged from 35 to 100% for *M. abscessus*, 50 to 100% for *S. epidermidis* and 88 to 100% for *B. subtilis*. After storage, culturability ranged from 1 to 60% for *M. abscessus*, 0 to 100% for *S. epidermidis* and 87 to 100% for *B. subtilis*. Recovery (numbers of organisms cultured from the filter) ranged from 0 to 5000 CFUs for all filters. Mean recovery before and after storage (R_b and R_a) is shown in Table 3 for each organism and recovery method.

TABLE 3
Numbers of CFUs Recovered From Filters Before Storage (R_b) and After Storage (R_a) for All Filters at All Loading Conditions (mean and standard deviation)

	R_b			R_a		
	Elution	RODAC-F	RODAC-B	Elutio n	RODAC-F	RODAC-B
<i>M. abscessus</i>						
Mean	80.5	78.4	31.5	1.9	2.2	0.5
SD	154	183	103	21	10.7	2.6
<i>S. epidermidis</i>						
Mean	135	473	121	13	24	5
SD	221	1243	823	43	89	20
<i>B. subtilis</i>						
Mean	201	1101	235	202	800	130
SD	467	1814	767	477	1520	498

Analysis of variance was used to determine those factors (Table 2) of greatest influence on culturability before and after storage (C_b and C_a) and survival (S). Mean values for C_a and C_b and adjusted least squares means for S are shown in Table 4. Those factors which were significant are shown in Table 5.

TABLE 4
Mean Culturability Before Storage (C_b), Culturability After Storage (C_a) and Survival (S) for All Factors in the Statistical Model

Factors and Levels	C_b (%)	C_a (%)	S		
			S(%) ^a	ALSM ^b	SE ^c
Organism					
<i>M. abscessus</i>	82.3	19.5	0.28	0.882	0.067
<i>S. epidermidis</i>	92	61	1.4	1.034	0.041
<i>B. subtilis</i>	98.9	98.3	523	1.87	0.031
Loading Flow					
45 L/min	88.5	54.3	6.8	1.212	0.032
85 L/min	94.2	61	15	1.313	0.043
Loading RH					
30% RH	86.5	52	11	1.273	0.038
70% RH	94.2	61	9.5	1.252	0.034
Recovery Method					
Elution	91	48	3.7	1.14	0.05
RODAC-front	96	60	2.6	1.099	0.042
RODAC-back	84	62	79.5	1.549	0.053

^aAdjusted least squares means re-transformed into the original scale, ^bAdjusted least squares means, ^cStandard errors in the least squares means scale.

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Multiple comparison tests indicated that culturability after storage (C_a) and survival (S) were both significantly different among the three organisms. As expected, both culturability and survival were highest for filters loaded with spores of *B. subtilis*. This bacteria is the least susceptible of the three test organisms to stress and desiccation. We initially expected that C_a and S would be least for filters loaded with *S. epidermidis* (because this organism was thought to be the most susceptible of the three to stress and desiccation), instead, filters loaded with *M. abscessus* showed the least culturability and survival (Table 4).

TABLE 5
Results of Analysis of Variance for Culturability After Storage (C_a)
and Survival (S)

Independent Variable	p-value	
	C_a	S
organism	<0.0009	<0.0009
loading flow	<0.0009	0.029
loading relative humidity	<0.0009	0.608
approval category	0.084	0.087
recovery method	<0.0009	<0.0009
model (nested in approval category)	NA	0.003
flow * loading relative humidity	0.690	0.900
organism * recovery method	<0.0009	<0.0009
approval category * recovery method	NA	0.009
organism*approval category	NA	0.032
organism * approval category * recovery method	NA	<0.0009
organism*loading flow	0.002	NA
organism*loading relative humidity	0.001	NA
organism*loading flow*loading relative humidity	0.423	NA

NA = not applicable; this variable was not included in the analysis of variance model

There are several possible explanations for the poor recovery of *M. abscessus* cells: 1) they did not survive generation, transport to and deposition on the filter as well as the other organisms; 2) they were not recovered from the filter media as efficiently as *S. epidermidis* or *B. subtilis* (perhaps due to their hydrophobic cell wall); 3) they did not survive as well on filter media as the other two test organisms or 4) the culturing conditions (i.e., media) inhibited growth in some manner.

The ability of organisms to survive aerosolization, transport to, and deposition on the filter may have influenced the number of viable organisms loaded on the filter, and therefore the number available for survival on filter media. *B. subtilis*, in spore form, is the most likely to survive the forces associated with aerosol challenge tests, while *M. abscessus* may have lost initial culturability due to desiccation and other physical factors. As shown in Table 4, culturability of *M. abscessus* prior to storage (C_b) was approximately 18% less than that of *B. subtilis*, and 10% less than for *S. epidermidis*.

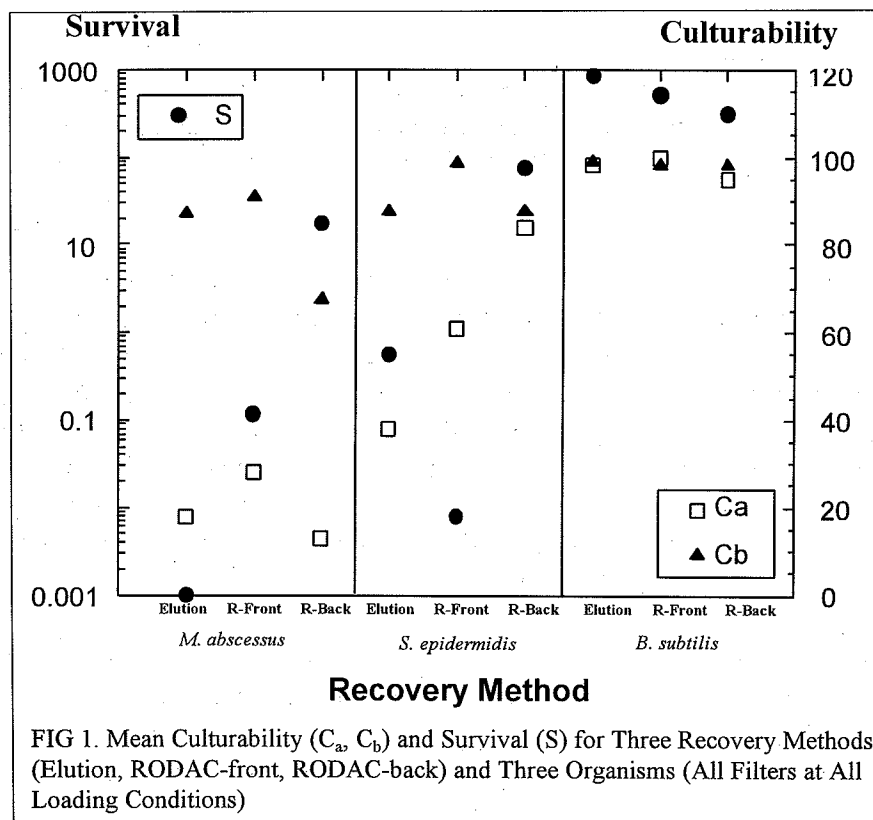
Survival of *B. subtilis* was much higher than expected (523%). Possible reasons for this may be: 1) more efficient recovery after storage than before or 2) organism replication during storage.

At this point, there is no evidence to support or refute either explanation; further research is needed.

Higher relative humidity during loading resulted in more filters with culturable organisms after storage. This may have been due to less organism desiccation during aerosol challenge. Higher flow during loading resulted in higher culturability after storage for all organisms, which was expected. More organisms will be captured at higher than at lower flow, if particle concentration and loading time are similar.

Culturability after storage and survival were greatest when organisms were recovered using RODAC sampling on the back of the filter (Table 4). Viable organisms on the back of the filter may be cause for concern because there may be increased potential for exposure to captured organisms. We suspect that fewer organisms were detected on the front of many filters because many of the disposable models had a relatively inefficient layer of material protecting the actual filter medium. Future experiments are needed to measure the presence and viability of organisms captured beneath this protective layer.

For all recovery methods, culturability after storage was highest for *B. subtilis*, followed by *S. epidermidis* and *M. abscessus* (Figure 1). Survival of *B. subtilis* was much higher than for either of the other organisms, using any of the recovery methods. There were no clear trends for the two recovery methods among the organisms, however. The effectiveness of each recovery method appears to differ among organisms, suggesting that preliminary evaluation of the methods used to recover a particular organism will be necessary in future experiments.



The results shown here exhibit a great deal of variability, largely due to lack of control over important test parameters. For example, because recovery was not expected to vary among organisms, recovery methods were initially developed using only *M. abscessus* at one challenge condition (45 L/min and 70% RH) on two filter types (McCullough et al., 1997a). During method development, filter type was found to influence recovery. The results shown here indicate that organism and challenge condition (in particular, relative humidity) are also significant factors in the degree of recovery from a filter.

Recovery measurements immediately followed filter efficiency tests. Organism loading was not controlled in these tests. Rather, the number of organisms loaded on a filter depended on efficiency of particle capture and the time it took to assess this efficiency. Very efficient filters (e.g., HEPA) capture more organisms than inefficient filters (e.g., NA) in the same period of time; the former require longer sampling times to assure adequate particle counts downstream of the filter. Better control of the number of organisms loaded on the filter, resulting in a similar R_0 for all filters, would have reduced the variability in recovery and prevented cases where $R_0 = 0$.

While these experiments provide information concerning organism culturability and survival over a five-day storage period at high relative humidity, they should be viewed as preliminary. Although few data are available on the nature and concentration of infectious aerosols in workplaces, the concentrations used in these experiments were probably higher than found in typical occupational settings.

SUMMARY

The objectives of these experiments were to determine 1) if cells are viable on filters following loading and 2) if organisms can be recovered from filters following storage for 5 days at 85% relative humidity. The results should be useful when designing future recovery and survival filter tests as well as when considering respirator handling, use and disposal procedures or policies.

Information obtained during this research can be utilized to improve future test protocols and reduce experimental variability. The experiments described here were initially designed to evaluate filter efficiency and did not control for organism loading. If tests had been designed to assure a uniform number of viable organisms on filters before storage ($R_0 > 0$, $C_0 = 100$) the data would have exhibited less variability. Design of future tests can take advantage of these results when selecting conditions which assure high culturability and survival. Further experiments are needed to evaluate additional factors which enhance or hinder survival, including environmental conditions and filter type. Tests are also needed to identify recovery methods which are most effective for specific filter types and organisms.

For organisms captured by filters to cause health effects the cells must remain viable and be released from the filter. These experiments did not address the release of organisms from filters during typical use situations. Anecdotally, we found that organisms were released from filters by handling and air movement. Culturable cells were found to be present on the outside surfaces of

gloves worn while handling loaded filters. Additionally, when clean air was passed through loaded filters (reversed in the test set-up), organisms could be sampled downstream of the filter. Further research is needed to evaluate whether organisms are released, both to surfaces and into the air, under typical handling and use conditions.

We have shown that culturable cells can be recovered from filters following a five-day storage period. This suggests that policies regarding reuse, handling and disposal of respirators and surgical masks should be carefully considered. If, as these results imply, bacteria can remain viable on filters for several days, wearers should be informed of the risks of reusing respirators and surgical masks. While concentrations used in these experiments were probably higher than those found in occupational settings, wearers should be trained to recognize when exposures might require immediate disposal of respirators. For example, respirators used in the isolation room of a potentially infected patient might be worn all day, while those used during respiratory therapy or autopsy procedures on an infected patient might be discarded immediately. Additionally, used respirators should be disposed of in accordance with policies for other potentially infectious waste.

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