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Mycobacterial Aerosol Collection Efficiency of Respirator and Surgical Mask Filters Under Varying Conditions of Flow and Humidity

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Collection efficiency was measured for a wide range of surgical masks and certified respirator filters using a 0.55-μm latex sphere aerosol at 45 L/min. Results were used to select representative filters and surgical masks for subsequent biological aerosol challenges. Collection efficiency of 16 respirator filters and 5 surgical masks was determined using a *Mycobacterium abscessus* aerosol at 45 and 85 L/min and 30 and 70 percent relative humidity. The bioaerosol was measured using two instruments: (1) an aerodynamic particle sizer, which detects both biological and nonbiological aerosols, and (2) a modified six-stage Andersen sampler, which detects only those aerosols able to replicate. Filter penetration of the latex and biological aerosols was highest and most variable for the surgical masks. Latex aerosol penetration through respirator filters ranged from 0.3 to 10 percent for dust/mist (DM) filters and from 0.09 to 3.4 percent for dust/fume/mist (DFM) filters; high efficiency particulate air (HEPA) filter penetration was always less than 0.01 percent. Median penetration of the biological aerosol was 2, 0.4, and 0.02 percent for DM, DFM, and HEPA filters, respectively. Higher flow resulted in higher penetration for all filters (as expected) and changes in relative humidity exerted only minimal influence on the collection of bioaerosols. The two sampling instruments gave similar values of filter efficiency; the total particle counter was generally less variable than the viable particle sampler. If the instruments detect similar size ranges and the aerosol consists largely of viable organisms, it may be possible to employ a nonviable particle sampler to assess biological aerosol penetration through filters. Selecting a surrogate test organism requires careful evaluation and may depend on geometric size, shape, density, and biological state. For assessment of filter performance, a worst-case test should simulate the most penetrating particle size and effects of relative humidity on both filter media and biological organisms. BROSSEAU, L.M.; MCCULLOUGH, N.V.; VESLEY, D.: MYCOBACTERIAL AEROSOL COLLECTION EFFICIENCY OF RESPIRATOR AND SURGICAL MASK FILTERS UNDER VARYING CONDITIONS OF FLOW AND HUMIDITY. APPL. OCCUP. ENVIRON. HYG. 12(6):435–445; 1997. © 1997 AIH.

The capture efficiency of respirator filter media challenged with nonbiological aerosols has been studied extensively, but few investigators have explored how respirator filters collect airborne microorganisms. Since present respirator certification tests employ nonbiological aerosols, it is important to establish that biological aerosols will be collected with similar efficiency and that environmental conditions of flow and relative humidity (RH) will not change their collection behavior in an unexpected manner.

Work by previous investigators has been carried out using a limited number of surgical mask and respirator filters. In most cases, a single biological aerosol has been employed, and the effect of changes in flow and RH on biological aerosol penetration has not been investigated in any detail. In only one case have viable and total particle measurement methods been directly compared.

One investigator used a *Bacillus subtilis* subsp. *niger* aerosol at 22.5 L/min to challenge three respirators and one surgical mask, which were sealed to a mannequin head. Aerosol penetration of 8, 22, and 33 percent was found for a half-facepiece high efficiency respirator, a disposable dust/mist (DM) respirator, and a surgical mask, respectively. Measurement of filter penetration was carried out using viable samplers (an AGI-30 for downstream and an AGI-4 for upstream measurements).[1]

A second study evaluated one manufacturer's surgical masks and disposable respirators using a *Mycobacterium abscessus* aerosol at 46 L/min. Penetration ranged from 1.4 to 2.8 percent for surgical mask and disposable DM respirators using a viable particle sampling method (Andersen cascade impactor), and from 2.1 to 3.2 percent using a total particle counter. A disposable dust/fume/mist (DFM) respirator demonstrated 0.04 and 0.12 percent penetration using the viable and total particle methods, respectively. Both sampling methods measured penetration of less than 0.01 percent for a disposable high efficiency particulate air (HEPA) respirator. A 0.55-μm latex sphere aerosol was used to challenge the masks and respirators as well, and was always about 1 percent more penetrating than the biological aerosol (except for the HEPA respirator, which continued to show less than 0.01% penetration).[2]

Finally, a third study challenged a surgical mask and a disposable DM respirator with a *Pseudomonas fluorescens* aerosol at 32 L/min and found penetration to be approximately 14 and 5 percent, respectively. A total particle sampler was used to measure upstream and downstream concentrations. Increasing the flow to 50 and 80 L/min caused penetration through the DM respirator to increase to 6 and 8 percent, respectively. Challenges with 0.8-μm corn oil particles showed penetration of 21 percent for the surgical mask and 10 percent for the DM respirator.[3]

All of these studies used bacterial aerosols with a similar aerodynamic particle size (about 0.8 μm) and similar flows (22.5 to 46 L/min). Two of the studies showed the same range of penetration for DM respirators (less than 5%); the surgical

masks were much more variable, ranging from 2 to 14 percent penetration.[2,3] In each of these studies, the findings were corroborated by challenges with nonbiological aerosol particles (latex sphere and corn oil). On the other hand, the study using *B. subtilis* found significantly higher penetration for all mask and respirator types, which suggests that the experimental protocol did not account for some important source of leakage or that the different samplers used to measure upstream and downstream concentrations introduced an unexpected bias. No corroboration with a total particle sampling method was carried out.[1]

None of these studies determined the effects of RH on the capture of biological organisms in filters. The effect of flow was evaluated in one of these studies for one respirator only. In only one of these investigations was a direct comparison made between a nonviable, total particle sampling method and a viable particle method.

This study was designed to expand on previous research and was carried out in three stages. In the first stage, a large number of respirator and surgical mask filters were challenged with a nonbiological, latex sphere aerosol. The results of these tests were used to choose a small number of representative filters, which were evaluated in the second phase by challenge with three biological aerosols at two flows and two RHs. Two samplers were compared: one that samples all particles (viable biological, nonviable biological, and nonbiological) and one that samples only viable biological particles. In the third stage, survival of organisms on loaded filters was evaluated. These experiments did not investigate the facial fit of respirators and surgical masks—all filters were sealed during testing.

Results from the first phase (latex sphere challenge tests) and partial results from the second phase—that is, results of challenges with one of the biological aerosols (*Mycobacterium abscessus*) and the comparison of the total and viable particle samplers—are described here. The two additional biological aerosol challenge experiments and filter survival tests are described elsewhere.[4,5]

Background

This research was designed to address three questions:

1. Do changes in RH and flow cause equivalent changes in filter penetration for both biological and nonbiological aerosols?
2. Can a sampler that measures all particles (i.e., viable biological, nonviable biological, and nonbiological) be used to measure viable biological aerosols employed in filter challenge tests?
3. How similar are the experimental results to those expected for *Mycobacterium tuberculosis*?

In the case of at least one highly infectious airborne disease (tuberculosis), federal agencies have agreed that respirators certified by the National Institute for Occupational Safety and Health (NIOSH) may be an appropriate means of preventing exposure.[6–8] These recommendations were made under the assumption that respirator filter media will collect nonbiological and biological aerosols in a similar fashion. Respirators are presently certified using challenges with nonbiological aerosols. Based on physical principles, filter collection of airborne biological organisms should be similar to that of nonbiological aerosols; however, no studies have evaluated this issue across the broad range of respirator filters. The effects of flow and RH on biological aerosol filter collection have also not been thoroughly addressed.

The Centers for Disease Control and Prevention (CDC) require that respirators used to control exposures to *Mycobacterium tuberculosis* must be able to filter 1-μm particles with $\geq$95 percent efficiency at 50 L/min.[6] For respirators certified under 30 CFR Part 11, only HEPA filters meet these criteria.[9] Since this research began, a new filter certification procedure, 42 CFR Part 84, was adopted by NIOSH.[10] All filters that meet the new guidelines will satisfy the aforementioned CDC criteria.

The new certification protocol requires filter preconditioning (prior to testing) for 25 $\pm$ 1 hours at 85 percent RH, which addresses the fact that prolonged exposure to moisture and heat may neutralize the filter media charge and thus reduce the collection efficiency of electrostatic filters.[11] In addition, a steady test flow of 85 L/min is required in the new tests (rather than 32 L/min used in the previous filter certification tests); this higher flow will generally result in lower collection efficiency, particularly for particles in the most penetrating range.[12] The biological aerosol challenges described here employed 24-hour preconditioning of filters at 85 percent RH and experimental flows of both 45 and 85 L/min to assess the effects of these worst-case test conditions on filter behavior.

To assure that comparisons of viable and nonviable particle samplers assess only the effects of the sampler (and not other environmental or test conditions) on the measurement of airborne concentration, it must first be established that each measures a similar particle size range. In addition, the aerosol must consist predominantly of viable cells; the presence of nonviable particles may skew or mask the measurement of viable particles (depending on their particle size). Through sampler modifications and careful preparation of the biological aerosol, both of these conditions were met for this research.[13]

The test organism, *Mycobacterium abscessus* (*M.a.*), was chosen as a surrogate for *Mycobacterium tuberculosis* (*M.tb.*) because the cells are physically similar (e.g., shape and dimensions), yet the former does not cause disease in immune-competent individuals and has a much shorter growth time.[14]

Respirators used in this study were certified under 30 CFR Part 11, which contains four tests for particulate respirator filters: dust, mist, fume, and high efficiency.[9] These four tests are used in combination to certify three respirator approval categories: DM, DFM, and HEPA. Surgical masks are not considered respiratory protection devices; rather, they are designed to prevent the wearer from expelling large particles into the environment (e.g., by sneezing).[15] As such, they do not undergo the rigorous testing specified by NIOSH for respirators. Surgical masks will be referred to as nonapproved (NA).

Methods

Challenge Tests with Latex Sphere Aerosols

The first stage of this research involved a set of experiments which determined the range of collection efficiencies of respirator and surgical mask filters by challenge with a nonbiological aerosol at 45 L/min. To ensure a wide range of efficiency within the four approval categories (DM, DFM,

HEPA, NA), a test aerosol with a particle diameter near the most penetrating particle size was selected.[16] These initial experiments allowed the selection of a small number of filters, from the full range of respirator filters and surgical masks available on the market, for further experiments with biological aerosols.

Collection efficiency was measured by challenging filter media with a nonbiological, 0.55-μm polystyrene latex sphere (PSL) (Seradyn, Indianapolis, Indiana) aerosol. Three replicates each of 75 NIOSH-approved, air-purifying respirator filters and 14 surgical masks from 20 manufacturers were tested (filter manufacturers and models are shown in Table 1).

A filter or surgical mask was prepared for testing by sealing to a metal plate which was covered with Scotch sandblasting stencil (3M Company, St. Paul, Minnesota). The mask or filter was sealed with hot glue directly over a central circular hole in the plate. Straps were removed from the filter to prevent interference with the seal. Valves, if present, were sealed with duct tape to prevent aerosol leakage through the valve. In the case of surgical masks and disposable respirators with filtering facepieces, the entire respirator or mask was sealed to the metal plate. Surgical masks with no internal structure were supported by a metal mesh. For respirator filter media in replaceable cartridges, the entire cartridge with filter was sealed to the plate. The plate was then loaded into the test system prior to each challenge test.

The test system was similar to that described by Brosseau *et al.*[14] The PSL aerosol was generated using a medical respiratory therapy nebulizer, the Misty Ox low-flow nebulizer (Medical Molding Corporation, Costa Mesa, California). The aerosol was then passed through a Kr-85 charge neutralizer (TSI, Inc., St. Paul, Minnesota) and combined with filtered and humidified dilution air. The aerosol traveled seven duct diameters to ensure adequate mixing prior to reaching the upstream concentration sampling port, and ten duct diameters before it encountered the filter. The aerosol that penetrated was sampled one duct diameter downstream of the filter. RH of the air stream was controlled to 50 $\pm$ 5 percent and was monitored before and after each test by measuring near the site of filter challenge.

A concentration of up to 200 particles/cm^3 was used to challenge HEPA and powered air-purifying (PAPR) filters; other filters were challenged with a concentration of 100 particles/cm^3. Filters for PAPR systems were tested at operating flow (113 L/min). All other filters were tested at a flow of 45 L/min.

The aerosol was measured with a direct-reading, particle counting and sizing instrument, the aerodynamic particle sizer (APS) (TSI, Inc.), which samples particles with an aerodynamic diameter of 0.5 to 30 μm at 5 L/min.[17] Alternating upstream and downstream concentration measurements were recorded. Filter efficiency was determined by averaging the respective concentration measurements and calculating the ratio of particles captured to those available for capture.

The results from the PSL aerosol challenges were used to select filters for the experimental phase utilizing bioaerosols. Prior to statistical analysis a log transformation was used to normalize the data and equalize the variance. Analysis of variance (ANOVA) was used to detect significant differences in the mean penetration between filters within each approval category (NA, DM, DFM, HEPA). Multiple comparison tests were then performed to divide each approval category into three classes: low penetration, medium penetration, and high penetration. Replaceable and disposable filters were randomly selected for subsequent bioaerosol tests. Where there were no significant statistical differences in filter penetration among filters in approval categories, those with the highest penetration (lowest efficiency) were selected.

Bioaerosol Challenge Tests

Bioaerosol tests were conducted with the same system described above for the latex sphere aerosol tests. *Mycobacterium abscessus* (*M.a.*) was maintained on Middlebrook-7H10 Agar (Difco, Detroit, Michigan). In preparation for aerosolization, organisms were removed directly from solid agar and mixed with deionized, filtered water in a Collison nebulizer (BGI, Waltham, Massachusetts) operated at 3 psi. During aerosolization, a magnetic stir bar in the nebulizer was used to mix the solution. Airborne concentrations of 0.5 to 2 particles/cm^3 were generated. *M.a.* was collected on the maintenance agar and incubated for 4 to 5 days at 36°C before colonies were enumerated in accordance with the positive hole conversion method.[18,19]

Filters were preconditioned for 24 $\pm$ 1 hours at 85 percent RH before being tested at two conditions of RH (30 and 70 percent) and two flows (45 and 85 L/min). Three replicates for each of 21 filter models from 13 manufacturers were conducted for tests at 45 L/min; a total of 63 tests were performed at each of the RH conditions. Three replicates of 12 filter models from seven manufacturers were conducted for tests at 85 L/min; a total of 36 tests were performed at each of the RH conditions. Three replicates of two PAPR filters from two manufacturers were conducted at operating flow at the two RH conditions; a total of 12 tests were performed. Table 2 shows the filters tested at each condition.

Alternating upstream and downstream measurements of aerosol concentration were made with two instruments. The first, an APS (described previously), detects all aerosols (both viable and nonviable). The second, an Andersen six-stage viable sampler (Andersen/Graseby, Atlanta, Georgia), is a cascade impactor which operates at 28.3 L/min and collects viable particles on agar plates with stage cut diameters ranging from 0.65 to >10 μm.[19] Two modifications were made to the Andersen for these experiments: (1) the upstream sampling flow was reduced to 1.5 L/min through the addition of a dilution system, and (2) the lower size limit was extended from 0.65 to 0.45 μm by adding a seventh stage. These modifications are described in more detail elsewhere.[13]

Samples collected using viable methods, such as the Andersen, are analyzed by counting colonies that result from organism replication. Organisms that have lost their ability to replicate will not be detected using this method. Although culturable organisms may be present prior to testing, many aspects of the tests, including sampling, may cause loss of viability and inhibit replication. This is of special concern when working with vegetative organisms such as *M.a.*, which are known to be susceptible to a variety of environmental factors. Therefore, measures were taken to preserve viability throughout our experiment. These included minimizing the stress on cells during suspension preparation (i.e., by not wash-

TABLE 1. Mean Efficiency and Standard Deviation of Surgical Masks and Respirator Filters Challenged with 0.55-μm Polystyrene Latex Spheres (n = 3)

Manufacturer	Model	Certification	Efficiency (%)	SD
*3M	1800+	NA	14.8	3.9
Technol	Cone	NA	16.2	5.7
*Johnson and Johnson	Surgine II	NA	12.0	2.1
*Anago	Natural	NA	81.0	4.5
Technol	Duckbill	NA	89.8	2.6
White Knight	Comfort+	NA	93.0	2.0
Johnson & Johnson	Sofloop	NA	93.5	1.1
3M	1812	NA	94.5	1.0
White Knight	Comfort Cone	NA	94.6	1.7
*Johnson and Johnson	Barrier	NA	94.8	0.9
Anago	Protector II	NA	95.8	0.9
*3M	1818	NA	96.0	1.5
White Knight	Laser	NA	96.6	1.1
Technol	Lazer	NA	98.0	0.3
*Moldex	2200	DM	83.4	0.9
*AO	R1050	DM	84.4	3.5
Moldex	2300	DM	85.1	1.3
*Willson	T10	DM	89.7	2.7
*AO	A1010	DM	90.6	2.9
*MSA	Type F	DM	91.3	3.1
MSA	457468	DM	91.8	1.1
Precept	65-3360	DM	92.1	2.3
Technol	DMR010	DM	93.0	1.1
Lab Safety Supply	13783	DM	93.2	0.1
MSA	96077	DM	94.1	1.3
3M	8715	DM	96.1	1.6
North	W7500-6	DM	96.6	0.9
3M	8710	DM	97.0	0.6
3M	1814	DM	97.2	1.0
Racal	Delta 1+	DM	97.4	0.3
Protech	F100	DM	98.6	0.9
AO	R90N	DM	98.8	0.7
*Lab Safety Supply	11293	DM	98.8	0.7
Draeger		DM	98.9	1.3
Uvex	1000	DM	98.9	0.6
3M	7258	DM	98.9	0.2
Willson	R10	DM	99.0	0.5
Glendale	F10-T	DM	99.3	0.3
Scott	642-D	DM	99.3	0.1
3M	2020	DM	99.5	0.3
AO	R30	DM	99.5	0.5
*Survivair	101000	DM	99.5	0.3
Moldex	8040	DM	99.7	0.3
*3M	9900	DM	99.7	0.2
Willson T20		DMF	96.96	1.90
*MSA	Type S	DMF	97.7	0.19
North	N7500-7	DMF	99.1	0.80
*Protech	F108	DMF	99.14	0.16
Lab Safety Supply	11294	DMF	99.24	0.13
Racal	Delta II	DMF	99.24	0.64
North	N7500-	DMF	99.27	0.48
AO	R56A	DMF	99.29	0.12
AO	R50A	DMF	99.43	0.30
Scott	642-F	DMF	99.48	0.07

TABLE 1. (Continued)

Manufacturer	Model	Certification	Efficiency (%)	SD
Survivair	104000	DMF	99.69	0.22
*3M	9925	DMF	99.69	0.14
*Willson	R11	DMF	99.72	0.13
3M	9920	DMF	99.79	0.01
Uvex	2020	DMF	99.91	0.05
Moldex	8030	HEPA	99.99996	0.00005
Protech	G-108	HEPA	99.99987	0.0002
Scott	642-H	HEPA	99.99975	0.0001
North	N7500-8	HEPA	99.99960	0.0003
3M	7260	HEPA	99.99938	0.0008
Willson	T40	HEPA	99.99935	0.0010
Glendale	23205	HEPA	99.99920	0.0012
Survivair	109000	HEPA	99.99915	0.0012
3M	7255	HEPA	99.99904	0.0011
Willson	2240	HEPA	99.99888	0.0016
*AO	R57B	HEPA	99.99885	0.0007
Lab Safety Supply	11299	HEPA	99.99879	0.0006
3M	2040	HEPA	99.99877	0.002
MSA	464035	HEPA	99.99843	0.0025
MSA	Type H	HEPA	99.99798	0.0022
3M	9970	HEPA	99.99782	0.0039
Willson	R12	HEPA	99.99667	0.0068
*Uvex 3010	3010	HEPA	99.94260	0.0098
3M	W-2953-T	DM-PAPR	99.59340	0.52
*Racal	Airmate	DM-PAPR	99.98510	0.0079
*North	9800-8	HEPA-PAPR	99.99850	0.0026
Racal	Airmate	HEPA-PAPR	99.99870	0.0013
Willson	R53	HEPA-PAPR	99.99980	0.0003
Protech	G-408	HEPA-PAPR	99.99980	0.0003
3M	W-3267	HEPA-PAPR	99.99990	0.00006
Willson	R-73	HEPA-PAPR	99.99990	0.0001
3M	W-3210-4	HEPA-PAPR	99.99990	0.0001
AO	RP3	HEPA-PAPR	100.0	0.00002
Survivair	108000	HEPA-PAPR	100.0	0.00002
Racal	P3	HEPA-PAPR	100.0	0
MSA	464807	HEPA-PAPR	100.0	0
MSA	H-Optifilter XL	HEPA-PAPR	100.0	0

*Filter selected for bioaerosol challenge.

ing the cells) and operating the nebulizer at as low a pressure as possible (3 psi).[13]

It is also important to prevent overloading the Andersen sampling plates. Each stage has 400 jets through which the viable particles pass before impacting on the agar. If the concentration is sufficiently high, two or more viable particles may pass through the same jet, impact at the same point on the agar, and appear as one colony. The positive-hole correction method is used to quantify and correct for the probability of multiple organisms passing through one jet.[18,19] While this correction may better predict the actual number of sampled organisms than the raw count, it introduces additional variability. For this reason, concentration, sample time, and dilution were adjusted to prevent heavy loadings.

Obtaining a representative sample of *M.a.* proved difficult. Often, very low or zero counts were obtained on the downstream Andersen samples for the HEPA filters. Conversely, high loadings were a problem on the downstream Andersen samples of several NA filters. When penetration did not fall within the expected parameters, filters were retested. This occurred when: (1) penetration was 0 percent or greater than 100 percent, (2) penetration measured by the two instruments (APS and Andersen) was very different, (3) penetration was unusually high and leakage was suspected, or (4) Andersen sampling plates were overloaded, had very low counts, or showed no organism growth. A total of 210 tests with *M.a.* were planned and 290 tests were performed; approximately 30 percent of the filters challenged with *M.a.* were retested.

The data from bioaerosol challenges were used to evaluate the effect of test conditions on filter penetration. Because the

TABLE 2. Filters Challenged with *Mycobacterium abscessus*

NA filters (surgical masks)
*3M 1800+
*3M 1818
Johnson and Johnson Surgine II
*Anago Natural
Johnson and Johnson Barrier
Dust/fume/mist filters
*MSA Type S
Racal Delta II
*Protech F108
*Moldex 3400
Willson R11
*3M 9925
HEPA
Uvex 3010
*AO R57B
**North 9800-9
Dust/mist filters
Willson T10
*Moldex 2200
AO/Cabot R1050
Lab Safety Supply 12293
*MSA Type F
Gerson 1710
*Survivair 101000
*3M 9900
**Racal Airmate DM

All non-PAPR filters were challenged at both RH conditions and 45 L/min; those indicated by an asterisk were tested at 85 L/min at both RH conditions. PAPR filters (**) were tested at 113 L/min at both RH conditions.

distribution of filter penetration was not normal (Figure 1a and b), a logit transformation normalized the distribution and equalized the variance of penetration (P) (Figure 2a and b):

$$\text{logit } P = \log[P/(100 - P)] \quad (1)$$

Penetration measured by the APS was compared with that measured by the Andersen using linear regression analysis; the latter was treated as the dependent variable. The statistical model did not include an intercept because the regression line was expected to pass through the origin.

The effect of three experimental parameters (approval, RH, and flow) on filter penetration was tested by ANOVA. Separate analyses were performed on measurements from the two sampling instruments. The statistical models included all factors and interactions (Table 3). Tukey's multiple comparison test was used to further investigate all significant variables and interactions of variables.

Results

Challenge Tests with Latex Sphere Aerosols

Measurements of collection efficiency from nonbiological (PSL) aerosol challenges were used to select filters for biological aerosol tests. ANOVA detected significant differences in the mean penetration between filter models in all categories except HEPA. Bonferroni multiple comparison tests were then used to divide the NA, DM, and DFM categories each into three classifications of efficiency, from which replaceable and disposable filters were randomly selected for bioaerosol challenge.[20] Within the HEPA filters, the lowest efficiency filters were chosen to be representative of the least protective filters in this class. (While MSA type H was initially chosen as a replaceable HEPA filter, that filter became unavailable and the AO R57B was substituted.) Table 1 lists all filter models tested and the mean and standard deviation of percent efficiency for each model. Filters selected for bioaerosol tests are indicated by asterisks.

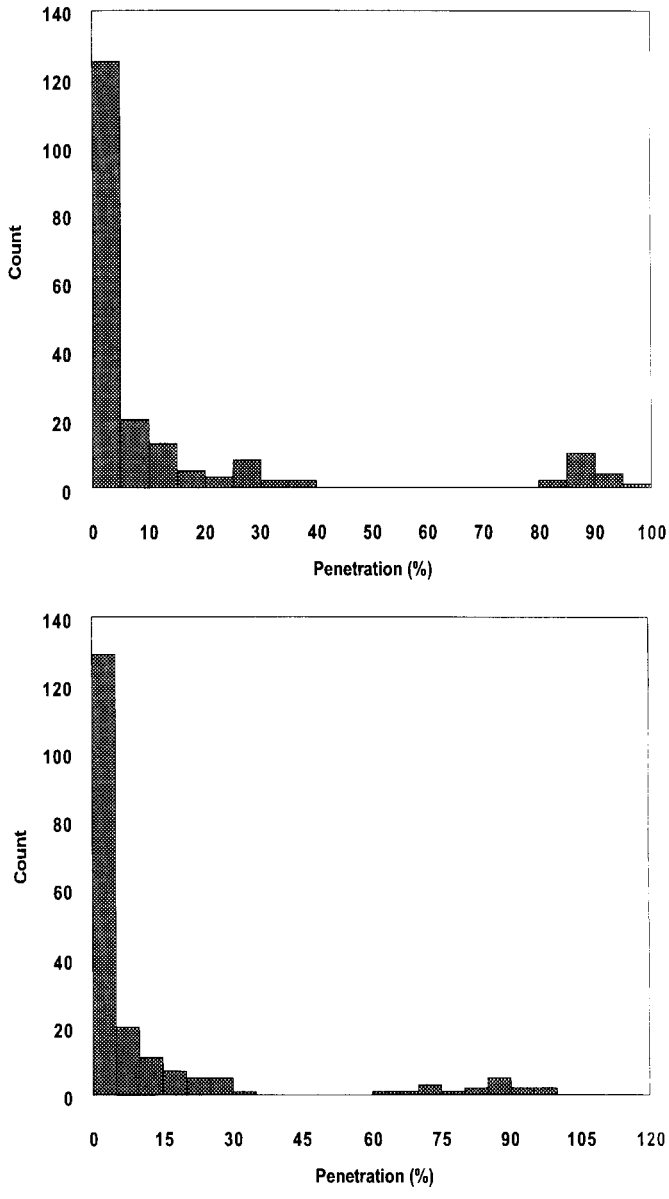

FIGURE 1. (a) Distribution of penetration measured by the APS, untransformed data. (b) Distribution of penetration measured by the Andersen sampler, untransformed data.

Bioaerosol Challenge Tests

The first statistical analysis compared measurements of penetration (P) made by the two sampling instruments (APS and

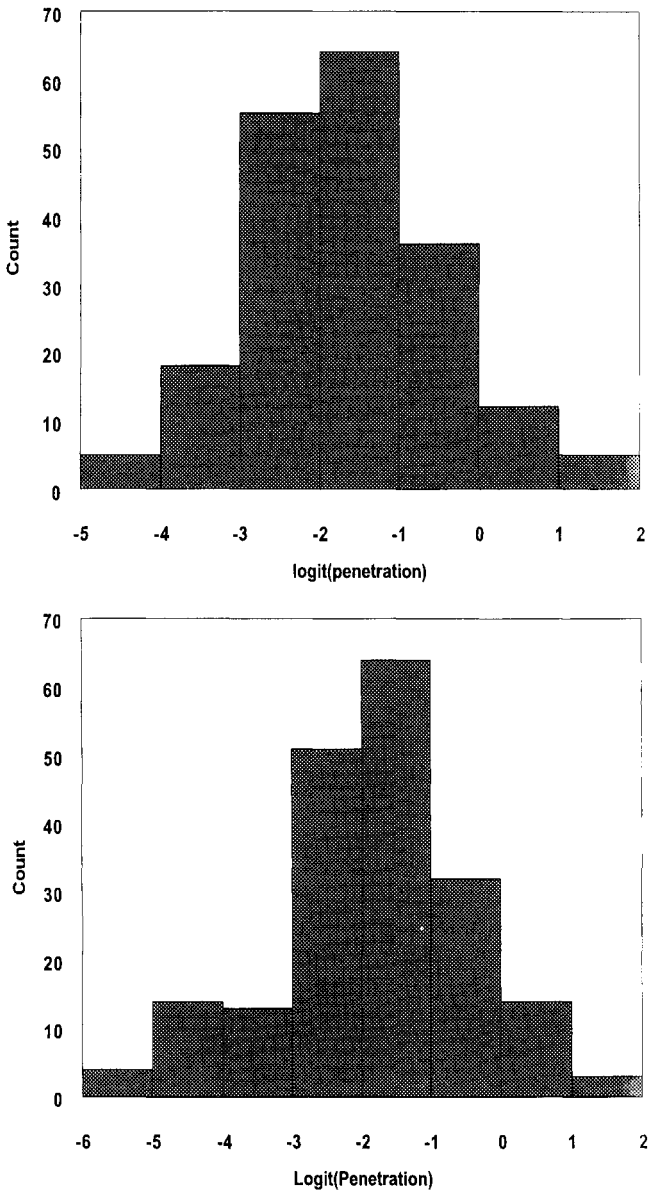

FIGURE 2. (a) Distribution of penetration measured by the APS, logit-transformed data. (b) Distribution of penetration measured by the Andersen sampler, logit-transformed data.

Andersen) using linear regression analysis. The final statistical model contained penetration measured by the Andersen as the dependent variable and three predictor (independent) variables: penetration as measured by the APS ($p < 0.0005$), approval category ($p = 0.0255$), and RH ($p = 0.022$). Flow was not a significant variable ($p = 0.522$). Approval category and RH were not expected to be significant variables. The removal of seven data pairs identified as outliers (studentized residuals $> |2.5|$) eliminated approval category ($p = 0.262$) and RH ($p = 0.184$) as significant variables and resulted in a regression equation of $P_{(Andersen)} = 1.076\ P_{(APS)}$ with an $R^2 = 0.978$. These results indicate a strong correlation between the measurements of penetration made by these two instruments (Figure 3).

TABLE 3. Variables and p Values Included in the ANOVA Model

Variable/Interaction	p Value
Penetration measured by the Andersen sampler as the dependent variable	
Approval	<0.0009
Model (nested in approval)	<0.0009
Flow	<0.0009
RH	0.059
Approval*flow	<0.0009
Approval*RH	0.479
Flow*RH	0.073
Penetration measured by the APS as the dependent variable	
Approval*RH*flow	0.063
Approval	<0.0009
Model (nested in approval)	<0.0009
Flow	<0.0009
RH	<0.0009
Flow*RH	0.755
Approval*flow	0.014
Approval*RH	0.520
Approval*flow*RH	0.440

The second statistical analysis utilized ANOVA to identify which test parameters were significant ($\alpha \leq 0.05$) in predicting filter penetration as measured separately by the APS and Andersen samplers. In each case, penetration was the dependent variable; predictor variables included in the statistical models and the corresponding p values are shown in Table 3.

Multiple comparison tests of approval found that penetration (as measured by both instruments) was significantly different among the four approval categories, with HEPA having the lowest penetration and NA filters having the highest penetration. These results were as expected.

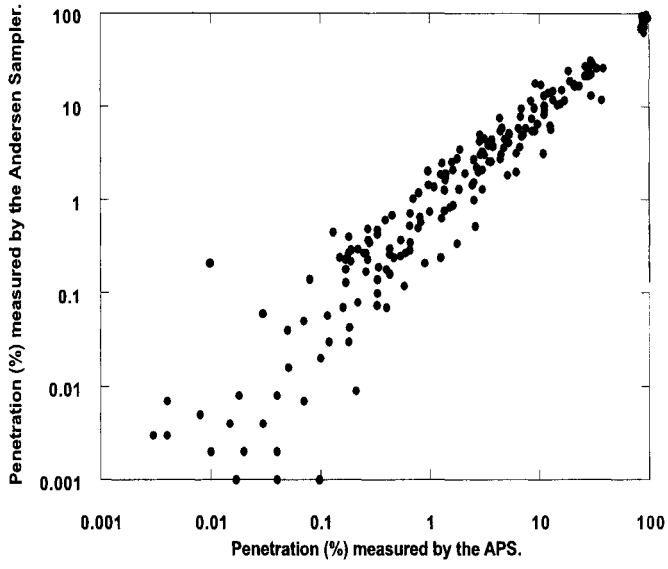

FIGURE 3. Penetration as measured by the APS and Andersen samplers.

TABLE 4. The Adjusted Least-Squares Mean (ALSM) in the Logit-Transformed Scale, the ALSM Retransformed into the Original Scale (ALSM*), the Geometric Mean (GM), and Geometric Standard Deviation (GSD) of Penetration (%) as Measured by the Andersen and APS Samplers

	Andersen				APS			
Factor	ALSM	ALSM*	GM	GSD	ALSM	ALSM*	GM	GSD
45 L/min	−2.38	0.41	0.81	22.1	−2.08	0.82	1.24	13.2
85 L/min	−1.86	1.37	2.24	9.1	−1.73	1.83	2.94	7.6
30% RH	−2.05	0.88	1.36	18.9	−1.79	1.59	2.22	10.8
70% RH	−2.19	0.64	1.01	15.6	−2.08	0.82	1.29	11.9
NA filters	−0.24	36.7	19.6	3.8	−0.19	39.5	23.1	3.6
DM filters	−1.67	2.11	1.91	5.3	−1.57	2.64	2.4	4.9
DFM filters	−2.56	0.28	0.35	7.1	−2.38	0.41	0.48	4.8
HEPA filters	−4.02	0.009	0.005	8.1	−3.48	0.03	0.03	4.4

Higher flow resulted in statistically higher penetration for all filters. Further analysis of the interaction of flow and approval, which was significant for both sampling methods, showed that only for HEPA filters was penetration statistically different at the two flows. Neither the surgical masks nor the DM respirator filters showed statistically significant changes in penetration from one flow condition to the other. The results with DFM respirator filters were equivocal: flow caused statistically significant changes in penetration using one sampling method (APS) but not the other (Andersen).

It was not possible to perform statistical tests on the interaction of flow, approval, and model, because the latter was a nested variable. We suspect that the lack of statistical significance at the two-way interaction level resulted from the variability introduced by the large number of different models (manufacturers) within each approval category. If the three-way interaction had been possible to evaluate, each model would probably have shown a statistically significant difference in penetration with changes in flow.

Differences in RH were not expected to lead to differences in biological aerosol penetration. Statistically, RH was not a significant factor when measured with the Andersen; however, for measurements made with the APS RH was a significant factor, with low humidity resulting in a slightly higher penetration (Table 4).

Filter penetration was highest and most variable for the nonapproved (surgical mask) filters, with a geometric mean penetration of about 22 percent [geometric standard deviation (GSD) of about 3.7] (Table 4). The DM respirator filters had a median penetration of about 2 percent (GSD of 5.1); the DFM respirator filters showed a median penetration of about 0.4 percent (GSD ranging from 4.8 to 7.1). The HEPA filters, as expected, had very low penetration, with a median of 0.02 percent (GSD ranging from 4.4 to 8.1). These results agree with those found in most previous investigations for similar-sized biological and nonbiological aerosols.[2,3]

Discussion

This research sought to answer three questions: (1) How is the penetration of bioaerosols affected by flow and RH? (2) Can an aerosol sampler that measures all aerosols be used to measure viable biological aerosols? and (3) How similar are these results to those expected if *Mycobacterium tuberculosis* had been the test aerosol?

Several particle filtration mechanisms are a function of velocity; changes in flow were expected to affect biological and nonbiological particles in a similar manner. It was expected that *M.a.*, based on its aerodynamic diameter of 0.7 μm, would be collected primarily by interception in filters relying only on mechanical filtration mechanisms.[16] There is no evidence that ambient RH plays a large role in nonbiological particle capture mechanisms; therefore, RH was not expected to significantly change bioaerosol filtration behavior.

In addition to flow and RH, the NIOSH approval category and the specific model of the filter were considered as independent variables in the data analysis. Penetration was expected to differ significantly among the four approval categories, because the certification criteria (or noncertification, for surgical masks) allow for considerable differences in filter penetration. Penetration among the various models was expected to differ as well because manufacturers employ a variety of filter media and designs.

Overall, the findings confirmed these expectations. Penetration among the four approval categories ranged from very high (NA) to very low (HEPA). For all filters, higher flow resulted in greater penetration. High efficiency filters were the only ones to demonstrate statistically significant changes in penetration between the two conditions of flow. However, the actual changes in penetration from low to high flow for all approval categories were always as expected: toward greater penetration. Examination of Table 5, which shows adjusted least-squares means from the statistical analysis, indicates that the penetration increased by 15 percent from low to high flow for surgical masks, 40 percent for DM filters, 60 percent for DFM filters, and 90 percent for HEPA filters.

While RH was found to cause statistically significant differences in penetration when evaluating all filters with the APS (but not with the Andersen), it is not clear that the small difference in penetration (0.8% less at lower humidity) has any practical or physical meaning.

The second goal of this research sought to evaluate the relationship between a sampler that measures the total aerosol (consisting of culturable biological, nonculturable biological, and nonbiological particles) and one that measures only particles containing culturable organisms. If these two sampler types can be shown to result in equivalent measures of filter efficiency, using a total aerosol sampler to measure bioaerosols would simplify tests. The use of such a sampler would also

TABLE 5. The Adjusted Least-Squares Mean (ALSM) in the Logit-Transformed Scale, the ALSM Retransformed into the Original Scale (ALSM*) for the Four Respirator Classes at the Two Flows as Measured by the Andersen and APS Samplers

	Andersen		APS	
Factor	**ALSM**	**ALSM***	**ALSM**	**ALSM***
NA, 45 L/min	−0.312	32.8	−0.233	36.9
NA, 85 L/min	−.0162	40.8	−0.138	42.1
DM, 45 L/min	−1.767	1.68	−1.678	2.06
DM, 85 L/min	−1.565	2.65	−1.458	3.37
DFM, 45 L/min	−2.735	0.18	−2.552	0.28
DFM, 85 L/min	−2.377	0.42	−2.21	0.61
HEPA, 45 L/min	−4.722	0.002	−3.856	0.014
HEPA, 85 L/min	−3.322	0.048	−3.109	0.078

reduce the variability associated with the sampling and analysis of culturable organisms, which results when cells are damaged during sampling or when corrections must be made for plate overloading.[18,19]

Although the two samplers employ different collection and analytical methods, we expected these instruments to produce equivalent measures of bioaerosol filter penetration. The strong linear correlation (R^2 = 0.978) between the two instruments' measures of penetration, with a slope near unity (1.076), indicates that the two samplers measure penetration equivalently. However, penetration measured by the Andersen was more variable (GSD = 18.1) than that measured by the APS (GSD = 11.5).

The higher variability in the Andersen measurements may be due to the analytic method, which relies on positive hole correction and optimal conditions for organism replication. It may be this variability which accounts for the substantial differences in penetration measured with the two instruments for the two flow conditions within the HEPA approval category (Figure 4).

Both sampling methods yielded similar measures of penetration at the two flows for the NA, DM, and DFM filters. The Andersen measurements of penetration, on the other hand, are quite different from those of the APS for HEPA filters. This difference is most marked at the 85 L/min test condition. Because HEPA filters are so efficient, it is difficult to collect organisms on the downstream side of the filter with an Andersen sampler without overloading the upstream samples. For example, to collect just one or two organisms downstream in the maximum sampling period of 15 minutes (necessary to prevent desiccation), a sufficient upstream concentration would cause overloading (more than 300 colonies/plate) in less than 10 seconds.

The final goal of this research considered the selection of an appropriate surrogate when the organism of interest cannot be employed in a laboratory setting. The definition of appropriate

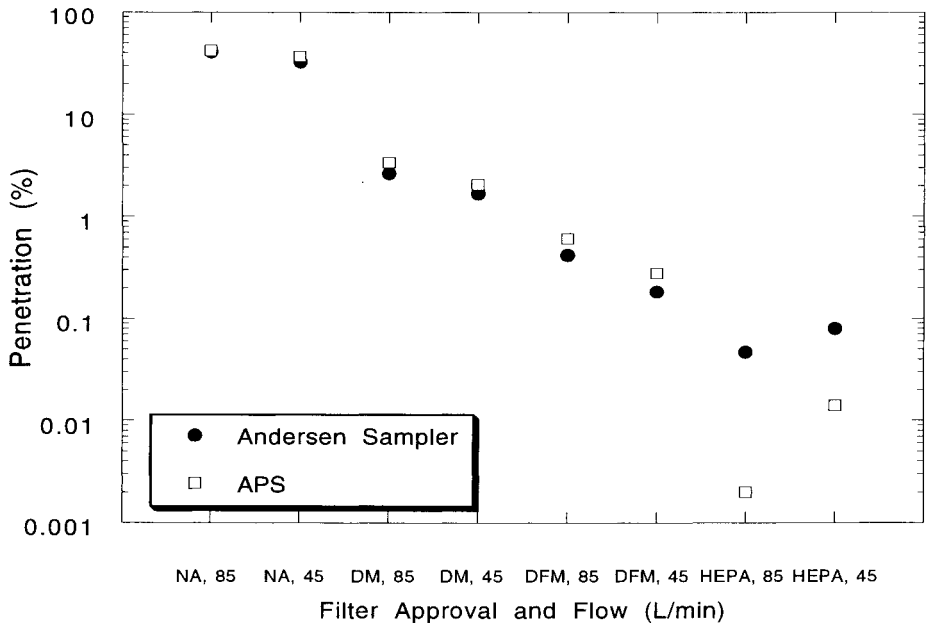

FIGURE 4. Filter penetration (adjusted least-squares means) at two flows measured by two samplers. Points represent the adjusted least-squares means retransformed from the logit-transformed scale.

surrogate depends on experimental goals. In this case, physical properties such as particle size and biological characteristics such as state (vegetative or spore) were considered when selecting an appropriate surrogate. *M. abscessus* was chosen as a surrogate for *M. tuberculosis* principally for its similarity in size, shape, and biologic state. Its relative nonpathogenicity and short growth time were important factors as well.[14,21]

Organism size is dependent upon geometric dimensions, density, and shape, all of which may influence the aerodynamic behavior of an organism.[16,22] The biological state determines an organism's viability, because a spore can survive adverse environmental conditions and still return to the vegetative state and reproduce, while vegetative cells are much more susceptible to changes in temperature and RH. Organism size may be related to biologic state as well, as spores may differ morphologically from vegetative cells of the same organism.[23]

Finally, tests of filter behavior when challenged with biological organisms should simulate worst-case conditions (i.e., those that will result in the highest penetration). While many argue that biological aerosols can only occur in the 1- to 5-μm range of droplet nuclei, single organisms of smaller aerodynamic diameter (which may be as small as 0.3 μm for *M. abscessus*) are more penetrating through filters and represent a better worst-case aerosol.[12,21] There is at present no evidence to suggest that single infectious bacteria cells cannot exist in the airborne state.

Conclusions

Overall, we found that changes in flow altered the collection of bioaerosols, with higher flow resulting in higher penetration. This result agrees with our expectations for filtration of nonbiological aerosols and reaffirms that the most important parameters of filtration of particles are their physical characteristics, such as aerodynamic diameter and shape. The biological state (i.e., viability) does not appear to influence the way in which particles are collected by a filter.

Additionally, this research demonstrated that, in certain cases, a total aerosol detector can be substituted for an instrument that collects culturable particles. A strong correlation was found between these two types of samplers. It should be kept in mind, however, that such comparisons are only valid when excess nuisance particles have been eliminated and viability loss has been minimized. In addition, the best comparison of these two types of samplers will occur when each measures an equivalent particle size range.

A surrogate test organism may be necessary if the target organism is hazardous, slow growing, or difficult to manipulate (such as *M.tb.*), but careful evaluation should be given to its selection. The geometric size, shape, density, and biological state will all be important factors when investigating filter behavior. Particle size may influence penetration; a worst-case test should use biological organisms near the most penetrating size of the filter media.

Filter efficiency tests provide information about how protective a respirator may be with respect to a bioaerosol, but do not evaluate the fate of the organisms following capture. Organisms that are captured by filters and remain viable may subsequently be released to the environment and cause health effects. A final phase of this research assessed the effects of storage on recovery and long-term viability; the results are discussed elsewhere.[5]

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