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Performance Aspects of Filtering Facepiece Respirators Against Ultrafine Inert and Biological Particles

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By

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ABSTRACT

Aspects of filter testing and performance of filtering-facepiece respirators (FFRs) were investigated with focus on particles in the ultrafine size range. Three specific aims were pursued in four related studies.

In the first study, the contribution to measured concentration made by the ultrafine fraction of the NIOSH respirator filtration test aerosols was theoretically modeled and tested. The most-penetrating particle size (MPPS) for N-type filters was observed in the ultrafine size range under laboratory conditions; also, ultrafine particles did not materially contribute to NIOSH's respirator filter test protocol.

The second study compared filter penetration and MPPS for N99 and N95 FFR's when challenged with ultrafine inert (NaCl) and biological aerosols (3 test viruses). The MPPS was observed in the ultrafine size for all respirators and test aerosols under conditions similar to the NIOSH filter test protocol. Inert particles penetrated filters similarly to virus particles, with several exceptions which were explainable and attributed to the physical and electrical properties of virus particles.

The third study developed and evaluated a method to compare physical and biological (viable count) filtration efficiency of two traditional respirators and one newly designed FFR with an iodinated treatment. The physical and viable virus penetrations were not found to differ for all three respirators; no killing effect was observed in virus particles which penetrated the filter medium.

An additional study assessed the appropriateness of nebulization to aerosolize virions for use in filter testing. An experiment was conducted to compare bioaerosol filter testing using nebulization to that of charge-reduced electrospray. The nebulizer protocol was

observed to be reasonably robust and it was concluded that nebulization is an appropriate method to use for aerosolizing virions for filter testing with appropriate protocols.

Overall, this dissertation contributed several key observations: (1) the definition of the "most conservative" test conditions for respirator filter testing was refined; (2) the understanding of factors which contribute to filtration differences of ultrafine biological and inert particles was increased; (3) a novel method was developed to differentiate physical and viable bioaerosol filter penetrations, and (4) the first practical comparison of nebulization and electrospray for aerosolizing virions was performed.

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EXECUTIVE SUMMARY

Recent interest in exposures to ultrafine particles (less than 100 nm) in both environmental and occupational settings led to questions as to whether the protocols used to certify respirator filters provide adequate attention to ultrafine aerosols. In the first study (**Chapter 2**), the particle size distribution of challenge aerosols were reviewed and the aerosol measurement method currently employed in the NIOSH particulate respirator certification protocol was evaluated for its ability to measure the contribution of ultrafine particles to filter penetration. Also considered were the differences between mechanical and electrically charged (electret) filters in light of the most penetrating particle size. The NaCl and DOP aerosols currently used in respirator certification tests were observed to contain a significant fraction of particles in the ultrafine region. However, the photometric method deployed in the certification test is not capable of adequately measuring light scatter of particles below approximately 100 nm in diameter. Specifically, 68% (by count) and 8% (by mass) of the challenge NaCl aerosol particles and 10% (by count) and 0.3% (by mass) of the DOP particles below 100 nm do not significantly contribute to the filter penetration measurement. Additionally, the most penetrating particle size for electret filters likely occurs at 100 nm or less under test conditions similar to those used in filter certification. It was concluded that the existing NIOSH certification protocol may not represent a "worst-case" assessment for electret filters because it has limited ability to determine the contribution of ultrafine aerosols, which include the most penetrating particle size for electret filters. Possible strategies to assess ultrafine particle penetration in the certification protocol were addressed.

In study 2 (**Chapter 3**), the performance of three filtering-facepiece respirators (two models of N99 and one N95) challenged with an inert aerosol (NaCl) and three virus aerosols (enterobacteriophages MS2 and T4 and *Bacillus subtilis* phage) – all with significant ultrafine components – was examined using a manikin-based protocol with respirators sealed on manikins. Three inhalation flowrates, 30, 85, and 150 L/min, were tested. The filter penetration and the quality factor were determined. Between-respirator and within-respirator comparisons of penetration values were performed. At the most penetrating particle size, over 3% of MS2 virions penetrated through filters of both N99 models at an inhalation flowrate of 85 L/min. Inhalation airflow had a significant effect upon particle penetration through the tested respirator filters. The filter quality factor was found suitable for making relative performance comparisons. The most-penetrating particle size for challenge aerosols was < 0.1 µm in electrical mobility diameter for all tested respirators. Mean particle penetration (by count) was significantly increased when the size fraction of <0.1 µm was included as compared to particles > 0.1 µm. The filtration performance of the N95 respirator approached that of the two models of N99 over the range of particle sizes tested (~ 0.02 – 0.5 µm). Filter penetration of the tested biological aerosols did not exceed that of inert NaCl aerosol. The results suggest that inert NaCl aerosols may generally be appropriate for modeling filter penetration of similarly-sized virions.

In study 3 (**Chapter 4**) the feasibility of a novel testing protocol that allows differentiating between the physical (total) and viable bioaerosol penetrations through respirator filters was investigated. Three respirator models—two conventional N95 filtering-facepiece respirators (FFR) used as controls and one P95 iodinated polymer FFR with antimicrobial properties—were challenged with aerosolized MS2 bacteriophage virus.

Physical ($P_{physical}$) and viable (P_{viable}) filter penetrations were simultaneously measured with the FFR sealed on a manikin at a constant inhalation flow rate of 85 L/min. Separate testing was performed on specially-manufactured P95 filter swatches with (a) no iodinated resin additive and (b) “high” amount of the additive to determine whether it influenced filtration behavior of the P95 respirator.

Bioaerosol collection on the N95 FFR filters fell in the range consistent with previous studies featuring about 2% penetration for MS2 and a peak around ~5%. The P95 iodinated polymer respirator was found to be highly efficient, attributed in part to the iodinated resin powder which in separate swatch tests was found to increase the filter collection efficiency. No statistically significant differences were observed between penetration values obtained for total and culturable viruses for the two control respirators. Similarly, no difference was observed for the iodinated respirator, which suggested that the microbial inactivation effect was of insufficient magnitude to be detected or was not present for viral particles that penetrated the filter. Possible “long-term” inactivation effect of the iodine-based additive on the viable viruses, which were captured on the filter over time, was beyond the scope of this study.

The novel testing protocol appears to be an adequate tool for evaluating respirators designed to protect against bioaerosol particles although further improvement may be considered with respect to the aerosolization method for viable microorganisms.

This conclusion led to conducting study 4 (**Chapter 5**) where aerosolization of MS2 bacteriophage virus by nebulization and charge-reduced electrospray were compared during testing of three filter media. Sample swatches were taken from a surgical mask, N95 filtering-facepiece respirator, and N100 respirator and utilized in repeated short duration (15

minute) penetration tests with MS2 bacteriophage aerosolized by nebulizer and electrospray. Evaluated were (1) the virus suspension preparation protocols, (2) resulting particle size distribution, count stability and variability, and (3) the ability to generate culturable MS2 virions. We observed that preparation of the electrospray suspension required additional purification and concentration steps and took more time than the nebulization protocol, but resulted in a much higher titer suspension. The nebulizer produced a polydisperse aerosol while that of the electrospray was relatively monodisperse, with a count peak at the mobility size of the single virion. Neither aerosolization method maintained constant count over repeated 15-minute filter tests; the nebulized aerosol particle count was 2.8 times as variable than the electrosprayed aerosol. Notably, no differences in filter penetration were observed between nebulized and electrosprayed MS2 aerosol. Electrosprayed dextrose particles—used as an inert aerosol comparator—were more penetrating than MS2 particles in two of the three filter samples—in part attributed to its dielectric properties. Both aerosolization methods produced culturable MS2 virion but the electrospray produced an airborne concentration ~20 times higher than nebulization. The electrospray appeared to produce a superior aerosol in terms of aerosol purity, stability, culturability when compared to nebulization. However, nebulization, when used in a repeated measures protocol provided similar filtration results. The findings of this study are expected to assist researchers in selecting appropriate generation methods when using viable virus- and bacteria-based challenge aerosols.

In summary, this dissertation expands and contributes to the scientific literature in several important ways. First, this dissertation at minimum refines and arguably redefines the “most conservative” scenario for use in respirator filter testing and certification. Second, this dissertation clearly defined the lower boundary of particle size detection of the existing

NIOSH certification protocol. Despite its *prima facie* importance to health and safety practitioners and manufacturers of respiratory protective equipment, this lower boundary has not previously been defined and illustrated in the literature. Third, this dissertation provided insight into several notable characteristics of ultrafine biological particles which may cause their filtration behavior to differ from inert surrogates. Fourth, a method was developed that can be used to rapidly assess the biocidal capability of treated respirator filters. Last, nebulization was compared to electrospray and shown to be a robust method for aerosolizing virions for filter testing.

LIST OF PEER-REVIEWED PUBLICATIONS

The results of this research have been published in the following peer-reviewed journal articles:

1. **Eninger RM**, Takeshi H, Reponen T, McKay R, Grinshpun SA (2008). What Does Respirator Certification Tell Us About Filtration of Ultrafine Particles? *J Occup Environ Hyg*; 5:286-295.
2. **Eninger RM**, Honda T, Adhikari A, Heinonen-Tanski H, Reponen T, Grinshpun SA (2008). Filter Performance of N99 and N95 Facepiece Respirators Against Viruses and Ultrafine Particles, *Ann. Occ. Hyg.* In Press, doi 10.1093/annhyg/men019.
3. **Eninger RM**, Adhikari A, Reponen T, Grinshpun SA (2008). Differentiating Between Physical and Viable Penetrations when Challenging Respirator Filters with Bioaerosols, *Clean – Soil, Air, Water: Special Issue on Bioaerosols*, In Press, doi 10.1002/clen. 200700198.
4. **Eninger RM**, Hogan, Jr CJ, Biswas P, Adhikari A, Reponen T, Grinshpun SA (2008). Electrospray Versus Nebulization for Aerosolization and Filter Testing with Virus Particles, DRAFT, submitted to *Aerosol Sci Tech*.

The full texts of the peer-reviewed publications are attached in Appendices A1 through A4. These four papers comprise the main body of this dissertation.

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HYPOTHESES, BACKGROUND, SPECIFIC AIMS

Hypothesis 1: The particles most likely to penetrate N-type filtering-facepiece respirators—which are in the ultrafine size range for certain laboratory conditions—are not adequately measured by the NIOSH respirator filter certification protocol.

Hypothesis 2: There are no filter penetration differences of similarly-sized aerosols of inert and biological particles in the ultrafine size range for both traditional and "biocidal" filtering facepiece respirators.

STUDY 1

Filtering-facepiece respirators (FFRs) are protective devices used in numerous workplaces to reduce airborne particulate exposures. The U.S. Bureau of Labor Statistics (BLS) in partnership with the National Institute for Occupational Safety and Health (NIOSH) estimated in 2001 that over 200,000 private establishments in the U.S. — totaling approximately 1.9 million workers — had utilized disposable particulate FFRs in the twelve months prior to being surveyed (NIOSH, 2003).

Particulate-filtering respirators marketed in the United States are subjected to performance certification prior to becoming commercially available. Certification ensures that respirators meet prescribed performance criteria intended to ensure a minimum level of user protection. Certification also results in an explicit stratification of respirator types and classes that aid the health and safety professional in selecting a level of protection appropriate for a specific hazard.

U.S. Government approval of respirators began in 1919 when the Bureau of Mines promulgated Approval Schedule 13 for self-contained breathing apparatuses (Held 1977). Approval requirements for other respirator types followed and certification requirements for particulate-filtering respirators were promulgated in 1934. With several modifications, the Bureau of Mines requirements eventually became the core respirator certification tests adopted by the newly-formed National Institute for Occupational Safety and Health (NIOSH) in 1972 (DOL 1983).

Currently, NIOSH certifies respirators in accordance with Title 42 of the U.S. Code of Federal Regulations, section 84 (42 CFR 84) (DHHS 1995). The regulations—adopted in current form in 1995—prescribe minimum performance requirements for respirator components and systems. Filtration efficiency of air-purifying particulate filters, a central focus of this dissertation, is certified under 42 CFR 84.181, *Non-powered air-purifying particulate filter efficiency level determination*. Respirators are certified in one of nine classes based upon three levels of filtration efficiency and three levels of resistance to filter degradation (see Table 1-1).

Respirator filtration efficiency is tested and certified for 95%, 99%, or 99.97% removal of challenge aerosol particles. These respirators are respectively labeled as “95”, “99”, or “100” class efficiency. Filter series is categorized as “N”, “R”, or “P”, based upon the type of aerosol used for testing. N-type filters are intended to protect workers from solid particulates and are tested against a mildly degrading sodium chloride (NaCl) aerosol; R-type filters demonstrate resistance to liquid particulates and are tested against a more highly degrading dioctylphthalate (DOP) oil aerosol; and P-type filters are highly resistant to degradation and are tested against DOP until filter efficiency is at its lowest level (DHHS 1995).

Since respirator users encounter a wide variety of aerosols under varying conditions, respirator testing is a combination of "worst case" and "very severe" conditions, i.e., a certified air-purifying FFR is intended to filter workplace aerosols as effectively (or more effectively) as it does when tested with the challenge aerosols under the NIOSH testing protocol (DHHS 1995). Research has shown mild filtration degradation when N-type filters are stored in high relative humidity conditions (Moyer and Stevens 1989a). Consequently, filters undergo preconditioning at 85% relative humidity and 38^{o}C for 25 hours prior to testing. The generated challenge aerosols are "charge-neutralized," which increases filter penetration when compared to charged aerosol particles (Moyer and Stevens 1989b). The test aerosols are intended to be at (or near) the assumed most penetrating particle size (MPPS) of 0.3 µm in aerodynamic diameter (Stevens and Moyer 1989, DHHS 1995, Hinds 1999b, Lee and Mukund, 2001). Since the respirator wearer's breathing minute ventilation can alter filter efficiency an airflow of 85 lpm (or 42.5 lpm for dual filter respirators) is used to represent a worker's inhalation at a high work rate (DHHS 1995, Stevens and Moyer 1989).

Respirator certification is intended to be a conservative test in order to assure a minimum level of filtration in a wide variety of workplaces, with differing aerosol characteristics, environmental conditions, and workloads. The current certification protocol, however, may not address the filter efficiency against ultrafine particles (with a diameter less than 0.1 micrometers = 100 nm), although this fraction is of special interest in environmental and occupational hygiene for several reasons (Vincent and Clement 2000). Due to high surface area per unit mass, ultrafine particles often have significantly different biological activity compared to larger airborne particles of the same composition (Donaldson et al. 2001). Patterns of respiratory deposition of ultrafine particles are not well-characterized and

there are no accepted particle-size selective criteria for their monitoring (Vincent and Chong et al. 2000, Clement 2001, Löndahl et al. 2006). Occupational sources of ultrafine particles are numerous. Some common sources involve combustion, such as diesel or aircraft exhaust, or welding fume generation (Vincent et al. 2000). Additionally, concern over appropriate protection against bioaerosols has increased in recent years. Microbial fragments have been observed in the ultrafine size range and it is hypothesized that viruses can be aerosolized in the ultrafine size range as droplet nuclei or single virions (Cho et al. 2005, Morawska 2006).

Recent developments in nanotechnology have resulted in a considerable interest in the health and safety aspects of engineered nanoparticles. These materials, used in a wide variety of commercial applications and products, include particulate materials < 100 nm in size which are engineered and manufactured with specific or unique properties. Risk assessment and management of engineered nanoparticles is still in infancy and guidance on exposure assessment and respiratory protection is limited (Oberdörster et al. 2005, NIOSH 2006). Although the ultrafine component of occupational aerosols rarely contributes in a major way to exposure in mass terms, it can pose a significant exposure in terms of particle count or surface area (Donaldson *et al*., 2001). Welding fume, diesel exhaust, and some biological airborne particles are examples of aerosols containing a considerable ultrafine fraction (Vincent and Clement, 2000).

Sources of engineered nanoparticle exposure are proliferating (Roco, 2001; Maynard and Kuempel, 2005). Potential health effects of nanoparticle exposure are of an increasing interest (HSE, 2004; NIOSH, 2004 and 2005a,b; Oberdörster *et al*., 2007). Additionally, biological aerosols such as airborne viruses and fungal fragments often belong to the ultrafine fraction (Reponen et al., 2001; Cho et al., 2005). Both severe acute respiratory

syndrome (SARS) and highly pathogenic influenza are caused by virions that can be smaller than 0.1 µm. Recent work by Morawska (2006) demonstrated that bioaerosol droplets can quickly dry in air to submicrometer and even ultrafine sizes and remain airborne for prolonged periods, thus representing a risk for infection.

Lack of practical and cost-effective technologies to evaluate exposures as well as uncertainty in the risks posed by ultrafine and nanoparticles has spurred an increasing awareness in the occupational health community and a corresponding demand for systematic research and guidance. The respirator certification protocols were developed and adopted prior to ultrafine and nanoparticle risk management possessed the importance it does today. Therefore, the purpose of the first study (**Specific Aim 1,Chapter 2**) was to evaluate the existing NIOSH respirator certification protocol from the perspective of its ability to provide users information on filtration in the ultrafine particle size range.

Specific Aim 1 addressed Hypothesis 1: Theoretically model and test in the laboratory the contribution to measured aerosol concentration made by the ultrafine fraction of the NIOSH respirator filtration test aerosols.

STUDY 2

Notably, a shift in the MPPS from ~ 0.3 µm to <0.1 µm under certain filtration conditions has been attributed to the electret properties of the respirator filter (Lathrace and Fissan 1986a,b), specifically to the polarization force affecting an electrically neutral particle and consequently changing the function of penetration versus particle size (Martin and Moyer 2000, Balazy et al. 2006a). This shift would alter what scenarios are considered "worst case" for the purpose of respirator filter certification.

Despite the need, there are limited data that can be utilized by health and safety practitioners for guidance in selecting respiratory protective devices for use with ultrafine aerosols, including airborne viruses. While some data on the filter performance of N95 respirators against nano-scale particles and MS2 virions have been recently published by this research group (Balazy et al. 2006a,b), no similar performance information is available for N99 respirators (which are increasingly used in occupational environments, including health-care settings). Therefore, the primary purpose of the second study (**Specific Aim 2, Chapter 3**) was twofold: 1) to evaluate size-fractioned filter penetration of N99 FFRs against inert and biological ultrafine aerosols at a wide range of inhalation flow rates – from 30 to 150 L/min; and 2) to compare respirator filter penetration values within and between filter classes, model, and challenge aerosol type (inert and biological). Also, this study served as a follow-up of our laboratory's previous work (Balazy et al. 2006a,b) that examined N95 respirators at 30 and 85 L/min. The data collected in this study was expected to provide respirator users with additional information for comparing filtration of N99 and N95 FFRs against ultrafine particles, including virions.

Specific Aim 2 addressed Hypotheses 1 and 2: Determine and compare the filter penetration and most-penetrating particle size for N99 and N95 filtering facepiece respirators when challenged with inert (NaCl) and biological aerosols (3 test viruses) with significant ultrafine fractions.

STUDY 3

More "exotic" respirator filter designs have recently become commercially available that are intended to control bioaerosol exposures with a focus on pandemic infectious disease.

It is known that bioaerosol exposure may pose numerous hazards in residential, occupational and ambient environments and possesses considerable public health significance. Bioaerosols are known to cause infectious diseases, allergic sensitization, and acute and chronic toxic effects (Burge 1990). Occupations with potential bioaerosol exposure may include—but are certainly not limited to—healthcare workers, wastewater and solid waste workers, biomedical researchers, workers in environments employing biomedical technology, farmers, veterinary workers, and food preparation workers (Lacey and Dutkiewicz 1994, Douwes et al. 2003).

Air-purifying respirators (APRs) are commonly used for reducing workplace exposures in situations where source-control is not present or is inadequate. However, governmental guidelines for respirator selection in occupational environments (if they exist) do not generally address bioaerosols or infectious agents. In the United States, guidance for selecting personal protective equipment (PPE), including respirators, is available only for some specific biological agents and environments with bioaerosol exposure, for example SARS (DHHS 2005), avian influenza (DHHS 2004, DOL 2006), and infectious or pathogenic agents in medical and laboratory settings (DHHS 2007). In addition, recommendations for selection of respiratory protection devices for mold exposure during remediation activities are available (NIEHS 2005). However, no unified strategy for selecting respiratory protection devices against bioaerosols has been developed and adopted in the US or worldwide (Rengasamy et al. 2004, Lenhart et al. 2004). Due to the anthrax attacks of 2001, the SARS outbreak of 2003, and the current threat of pandemic influenza, international organizations, governments and industry are increasingly focused on the development and performance evaluation of disposable and reusable respiratory protection

devices that would be efficient against airborne biological particles (mostly viruses and bacteria). The need for billions of disposable respirators for workers and general population in the event of pandemic or terrorist attack has been recently recognized in many countries, which began their stockpiling. Given the tremendous resources involved in the preparedness programs nationally and internationally, it is especially important to determine the efficiency of selected respirators against the bioaerosol agents of concern.

Investigators assessing the collection efficiency of respirator filters have observed that (1) inert aerosol particles (non-biological surrogates such as sodium chloride) sufficiently mimicked filtration of bioaerosol particles of similar aerodynamic diameter; and (2) direct reading instruments used for measuring the filter penetration produced similar results when compared to culture-based methods for a given bioaerosol (Chen et al. 1994, Willeke et al. 1996, Brosseau et al. 1997, McCullough et al. 1997, Wake et al. 1997, Qian et al. 1998). These investigations were performed primarily using challenge bioaerosol particles of around 1 µm or larger. Two other studies addressed smaller particles while comparing the respirator filter efficiency of bioaerosol challenges such as MS2 bacteriophages versus inert (non-biological) aerosol particles in the ultrafine fraction (<0.1 µm) (Richardson et al. 2006, Eninger et al. 2008b). The viral particle size range addressed in these studies includes the hypothesized most-penetrating particle size identified for respirators with electrically charged ("electret") filters when challenged with neutral aerosols (Balazy et al. 2006a,b, Eninger et al. 2008b).

Bioaerosols possess an added layer of complexity when compared to inert particles with respect to respirator selection (Rengasamy et al. 2004, McCullough and Brosseau 1999). Microbial transmission, viability, proliferation, and pathogenicity must all be taken into

account. Health effects from bioaerosols may relate to toxic components—which are present whether a microbe is viable or not—such as bacterial cell wall lipopolysaccharides (Douwes et al. 2003). Alternatively, health effects may relate to infectious potential or toxin production during infection. Also, the infectious dose of a given biological agent spread in aerosol form may be very low, which makes a hazard disproportionate to its airborne mass or count concentration and precludes the use of a traditional exposure limit.

Among the approaches recently introduced for improving the effectiveness of respiratory protection against bioaerosols, one is based on adding biocidal components to the filter medium in order to inactivate viable microorganisms. These respiratory protection devices (known as “antimicrobial” or “biocidal” respirators) aim at inactivating either microorganisms penetrating through the filter (“instant killing” effect) or those captured by the filter (effect occurs over the time of filtration) (NAS 2006). Filter media with additives such as halamine, silver or titanium-based nanomaterials, or iodinated powders have a potential for utilization to increase durability, protection, and aid in filter decontamination (Sun and Xu 1998, Sun and Sun 2003, Tessier et al. 2005, Voight et al. 2006, Heimbuch and Wander 2006, Li et al. 2006, Luo and Sun 2006). Adequate methodologies and protocols are needed to evaluate the performance of these newer, more “exotic” respirator filters with a specific focus on testing their antimicrobial capability. At present, professionals are debating whether assessing filtration specifically for the viable microorganisms should become an appropriate part of the respirator performance evaluation requirements (NAS 2006).

To fully assess the total particle filtration efficiency and antimicrobial effect of a bioaerosol-filter interaction, an ideal protocol should differentiate between physical (often

referred to as "total") filtration efficiency ($\eta_{physical}$) and viable filtration efficiency (η_{viable}). For a filter to exhibit biocidal effect, η_{viable} must exceed $\eta_{physical}$.

Existing respirator performance testing standards in the US do not fully address $\eta_{physical}$ and η_{viable}. According to the protocol of the Center for Devices and Radiological Health (CDRH) within the US Food and Drug Administration (FDA), surgical masks and respirators classified under 21 CFR 878.4040 (DHHS 1988) and intended for disease prevention are evaluated by undergoing two tests (ASTM 2004). The first test uses 0.1 µm polystyrene latex (PSL) spheres and serves to measure the total (physical) particulate filtration efficiency (ASTM 2003). The second test utilizes aerosolized *Staphylococcus aureus* bacteria and aims at measuring the viable filtration efficiency (ASTM 2007). However, because the challenge aerosol particle sizes in the above two tests differ by an order of magnitude—0.1 µm compared to ~1 µm—the resulting filtration efficiency measures are not comparable and, even when coupled, are not informative in assessing the performance of respirators with claimed antimicrobial properties. As mentioned previously, NIOSH certifies the performance of respirator filters based on measuring the total filtration efficiency of two non-biological challenge aerosols: sodium chloride, and dioctylphthalate (DOP) each with a mass median aerodynamic (MMAD) size of ~0.3 µm. Thus, the NIOSH certification protocol does not assess viable filtration efficiency.

No existing respirator test methodology/protocol differentiates between $\eta_{physical}$ and η_{viable}. Therefore, the purpose of the third study (**Specific Aim 3, Chapter 4**) was to develop and test a protocol to enable differentiating physical and viable respirator filter penetrations when exposed to a bioaerosol. The purpose of this study was to develop and assess the feasibility of a test protocol that integrates common instruments and approaches and enables

the above-mentioned differentiation when respirator filters are challenged with viable bioaerosol particles, including single virions representing the most penetrating particle size for electret filters. A practical method for such an evaluation would allow an appropriate evaluation of respirators with claimed antimicrobial properties.

Specific Aim 3 addressed Hypothesis 2: Develop a method to determine and compare the physical (in terms of particle count) and biological (in terms of viable count) filtration efficiency of two traditional respirators and one newly designed filtering facepiece respirator with an iodinated treatment of the filter structure.

STUDY 4

In the course of studies 2 and 3, it was identified that the nebulizer protocol used to aerosolize biological particles may create an aerosol with properties that could cause spurious results in our filter testing. This shortcoming could also have broader implications because numerous studies utilize the Collison jet nebulizer for aerosolizing both inert and microbial aerosols. It is used for the wet dispersion of particles to form droplets or droplet nuclei through evaporation of a desired material suspended in a solute (May 1973). The Collison nebulizer is a pneumatic or “air blast” nebulizer and uses compressed air flow to draw liquid from a reservoir using the Bernoulli effect, breaking the solution into droplets. The larger droplets impact the vessel wall from which they are drawn while smaller droplets are entrained in an exit air flow where they may be dried to droplet nuclei (Mercer 1968, Lefebre 1989).

Nebulization is a common and widely used method for aerosolizing biological aerosols such as bacteria (Jensen et al. 1992, Grinshpun et al. 1997, Foarde et al. 1999, Johnson et al.

1999, Griffiths et al. 2001, Li and Lin 2001, Mainelis et al. 2005), fungi and fungal fragments (Lin and Li 2003), and viruses (Agranovski et al. 2005, Balazy et al. 2006a, Kim et al. 2007). Nebulization is a relatively inexpensive aerosolization technique and does not require extensive user training or trial and error to obtain correct and reliable operation.

Nebulization has been widely applied to aerosolize inert and biological particles for respirator filter testing. Initially performed using particles > 0.3 µm because aerosol measurement was often made using optical techniques, one purpose of this testing was to assess whether differences existed between the filtration behavior of biological aerosols and similarly sized inert aerosols. Reviewed by Rengasamy et al. (2004), these studies were summarized previously. Eventual development and commercial availability of differential mobility analysis (DMA) and scanning mobility particle sizing (SMPS) spectrometry enabled size-fractioned filter penetration testing at particle sizes in the ultrafine size range, < 0.1 µm (Wang and Flagan, 1990).

Nebulizers produce a polydisperse aerosol which can include droplet nuclei containing biological particles in the ultrafine size range. In our laboratory it has been used to aerosolize MS2 bacteriophages to test respirator filters and to compare ultrafine inert and biological particle penetration (Balazy et al. 2006a, Eninger et al. 2008b, Eninger et al. 2008c). However, several drawbacks of nebulization for aerosolizing ultrafine biological aerosols have been identified. First, the presence of contaminant particles—dried solutes and biological fragments in the ultrafine size range resulting from the preparation and purification of the nebulizer suspension—may mask the biological particle of interest in terms of size-fractioned particle count (Hogan et al. 2004, 2005). This phenomena is not as prominent with larger biological particles because the solute and contaminant particle sizes are much smaller

than the particle of interest. A recognizable peak at the nominal particle size can be obtained with nebulization for larger biological particles.

Second, MS2 preparation and nebulization may produce microbial particle aggregates, as has been observed by Hogan et al. (2004, 2005). Aggregates can bias culture-based filtration measurements because the filtration behavior is influenced by particle size. Virion aggregates, filtered based upon their bulk size and shape, may be broken up during sample processing and counted using culture-based methods. Previous work from this lab with a nebulizer-based aerosol protocol suggested that MS2 aggregates did not contribute materially to the aerosol particle count (Balazy et al. 2006a). Balazy did not observe MS2 virion aggregates in a sample of prepared suspension viewed by electron micrograph. However, the studies by Hogan et al. (2004, 2005) directly observed MS2 virion aggregates on filter substrates and by using size-selective, culture-based methods, although a higher titer of bacteriophage than that in Balazy's study was used.

Lastly, nebulization of MS2 virions was observed to produce a time-varying particle size distribution (Hogan et al. 2005), which is not desirable for filter testing. The duration of nebulizer testing was not provided in the cited study, but this is a known characteristic of certain nebulizer designs. Most of the sprayed solution flows back to the nebulizer reservoir. Depending upon the chosen solute, a fraction will evaporate over time causing the solution to become more concentrated (Chen and John 2001). Techniques to minimize this include using a large solute reservoir, cooling the nebulizer and presaturating supply air, and delivering the solution at a constant mass flow rate (Liu and Lee 1975, DeFord et al. 1981).

Because of potential interference from contaminants and aggregates, it was realized that nebulization may not be the ideal way to aerosolize ultrafine biological aerosols for respirator

filter testing, particularly if the particle size of interest was in the ultrafine range. In theory, some differences should exist between the filtration behavior of smaller (~ 1 µm and smaller) inert and biological particles under certain circumstances. For a neutral or Boltzmann charged aerosol being filtered by electrically charged ("electret") media, the particle's dielectric properties influence the dielectrophoretic or polarization force between the charged fiber and the particle. In this case, filtration efficiency should be higher for particles with high dielectric constant. Materials used as inert aerosols in filter testing often possess low dielectric constants (ε_p) such as NaCl ($\varepsilon_p \sim 6$). Virions of similar size to MS2 bacteriophage have estimated dielectric constants of greater than 55 (Aristides et al. 2007, Lepizco-Encinas and Rito-Palomares 2007).

Charge-reduced electrospray is a promising aerosol generation method that appears to avoid the discussed shortcomings of nebulization. Electrospray aerosolizes a liquid using Coulombic repulsion from an electrical voltage potential between a capillary tube and a ground plate (Fenn et al. 1990). It can produce an ultrafine virion aerosol with a clear peak at the nominal virion diameter without aggregates, appreciable dried solutes and microbial fragments and without the shear stress associated with nebulization. This has been shown using MS2 bacteriophage (Thomas et al. 2004, Hogan et al. 2006). Hogan et al. observed that viable MS2 bacteriophage could be aerosolized using charge-reduced electrospray and that measured particle counts at the nominal virion diameter were linearly-related to the virus titer of a prepared liquid suspension.

Therefore, the purpose of Study 4 (**Corollary to Specific Aim 3, Chapter 5**) was to investigate and compare practical aspects of aerosolizing MS2 bacteriophage via nebulization and charge-reduced electrospray for filter testing applications. Each aerosolization method

was evaluated and compared in the following areas: (1) viral suspension preparation protocols; (2) resulting aerosol concentration, particle size distribution and stability over short time periods (15 minutes); (3) viability; and (4) filtration behavior.

Corollary to Specific Aims 2 and 3: Assess the appropriateness of nebulization as a method for aerosolizing virions for use in filter testing.

SPECIFIC AIM 1

Theoretically model and test in the laboratory the contribution to measured aerosol concentration made by the ultrafine fraction of the NIOSH respirator filtration test aerosols.

The purpose of the this study was to evaluate the existing NIOSH respirator certification protocol from the perspective of its ability to provide users information on filtration in the ultrafine particle size range.

2.1 REVIEW OF NIOSH PROTOCOL FOR TESTING FILTRATION AND METHODOLOGY FOR EVALUATION OF THIS PROTOCOL

2.1.1 Overview

The parameters of a respirator filtration test critically affect the findings on the respirator performance and consequently the practical implications of the test outcome. Four primary determinants of aerosol filtration are the challenge aerosol characteristics, the respirator filter characteristics, the aerosol measurement method, and the test conditions (see Figure 2-1). Challenge aerosol characteristics include (but are not limited to) the physical state and density of particles, the particle size distribution, and electrical charges. Respirator filter characteristics include the filter substrate, surface area, thickness, fiber diameter, surface density, and fiber electrical charge (for electret filters). Aerosol measurements are generally concerned with the particle count, surface area, mass (or related characteristic such as light scatter); the measurement method is based on a specific principle, such as gravimetry

or photometry, and characterized by the limit of detection and other factors. Test conditions are characterized by the temperature, relative humidity, flow, filter preconditioning, loading, test duration, number of test replicates, and procedures chosen for mounting/sealing a filter in a test chamber. Three of these parameters—challenge aerosol particle size distribution, filter electret properties, and challenge aerosol measurement method—were evaluated in this study in order to define the lower boundary of detectable particle size associated with the NIOSH filtration certification test.

The methods to perform this evaluation are summarized here. The physical characteristics of challenge aerosols used in the NIOSH respirator certification protocol were reviewed. Next, the size-fractioned light-scatter of the NIOSH test aerosols was modeled and aerosol measurement methods for determining filtration efficiency were evaluated with respect to their ability to detect the contribution of all particle sizes present. Finally, strengths and shortcomings of the existing certification aerosol detection methods were reviewed in light of published findings about the most-penetrating particle size (MPPS) for electret filters.

2.1.2 Challenge Aerosols

First, an ideal aerosol for utilizing in testing respirator filtration should be safe to use, easy to generate, measure, maintain a stable challenge concentration, and replicate at different laboratories. Second, its penetration through respirator filters should represent a "very severe" or "worst case scenario" relative to the expected workplace aerosol contaminants. Third, it should be as degrading or more degrading to a filter material than workplace aerosols. No single challenge aerosol fulfills all of these requirements, and filter

testing for non-workplace contaminants and environments (i.e., military applications) may need to differ.

The aerosol utilized by NIOSH to evaluate respirators for use against solid particles is sodium chloride (NaCl) (DHHS 1995). The test aerosol is required to have a 75 ± 20 nm count median diameter (CMD) and a geometric standard deviation (GSD) of less than or equal to 1.86. Based upon a density of 2.13, it has a mass median aerodynamic diameter of 347 nm (National… 2006a). The aerosol that NIOSH utilizes to evaluate respirators against liquid particles is dioctyl phthalate (DOP). This oil-based aerosol was chosen for its degrading properties and is required to have a CMD of 165 ± 20 nm and a GSD < 1.6. Based upon a density of 0.986, its MMAD is 356 nm (National… 2006b). The characteristics of both challenge aerosols are summarized in Table 2-2 and Figure 2-2. Logarithmic distributions theoretically have no upper limit; we assumed an upper bound of 1 µm. In practice, this is a reasonable estimate of the largest particle sizes observed in the certification tests. In the literature, the NIOSH challenge aerosol is often referred to as "0.3 µm in size", which technically means the mass median aerodynamic diameter discussed here. The above indicated aerodynamic diameter was selected based upon a most penetrating particle size (MPPS) predicted by single fiber filtration theory of mechanical filters, which is applied to respirator filters undergoing the NIOSH testing program (DHHS 1995, Hinds 1999b).

The charges carried by the challenge aerosol particles influence filter penetration. The NIOSH challenge aerosols are equilibrated to a bipolar Boltzmann charge distribution, which results in zero net charge. This is commonly referred to as a "charge-neutralized" aerosol. Since individual particles of a charge-neutralized aerosol may carry a positive or negative charge, this is an example of a "very severe" rather than a "worst case" test condition. A

worst case condition, although not as applicable to workplace aerosols, occurs when both individual particles and the aggregate aerosol possess no net charge (Moyer and Stevens 1989b, Hinds 1999a).

2.1.3 Aerosol Measurement Method

An ideal aerosol measurement method for testing filters should be rapid, accurate and reproducible, maintain calibration, and cover an appropriate particle size range that includes the most penetrating particle size (MPPS) for all tested filter materials. It seems that none of the currently available measurement methods meet all the above criteria.

The NIOSH testing protocol utilizes two forward-light scattering photometers to simultaneously measure aerosol concentrations before ("upstream") and after ("downstream") the respirator. Photometers measure the amount of light scattered by an assemblage of aerosol particles, which for certain particle sizes is proportional to aerosol mass (Gebhart 2001). For a given wavelength of incident light (λ), scattering angle, and particle index of refraction, the flux of scattered light by an assemblage of particles (R) is proportional to concentration and depends on the particle size distribution according to the following relationship (Gebhart 2001):

$$R = c_n \int_0^\infty f(d_p) P_\lambda(d_p) d\left(d_p\right)$$

Here c_n is the particle number concentration, $f(d_p)$ is the particle size distribution probability density function, and P_λ is the single-particle flux of scattered light. The NIOSH filter testing protocols utilize a specific incident light wavelength, particle indices of refraction, and scattering angle, which are the same for upstream and downstream

measurements. Therefore, we will focus on the effect of particle size on the measurement results.

The certification testing deploys a TSI model 8130 Automated Filter Tester (TSI, Inc., St. Paul, MN), which embeds two forward-scattering laser photometers for upstream and downstream particle concentration measurements. Scatter from the 780 nm wavelength laser light is measured at 45° of incidence. For particle physical diameters less than approximately half the incident light wavelength (½λ), scatter is proportional to d_p^6 and increases monotonically with increasing particle size (Gebhart 2001). For the NIOSH challenge aerosol, this includes particles up to ~380 nm in physical diameter. For aerosol particles larger than 380 nm (>½λ), scatter is overestimated by this relationship, and more sophisticated methods are required. As the aerosol concentrations upstream and downstream of the filter are indicated by the photometer output voltages, percent filter penetration is calculated as the ratio of these concentrations, corrected for a zero background, multiplied by one-hundred:

$$P = \frac{C_{down}}{C_{up}} \times 100\%$$

Note that P, which represents penetration in this equation, is separate and distinct from the earlier defined P_λ.

Photometry is extremely useful as it provides a rapid way to estimate upstream and downstream aerosol concentrations when testing the filter performance. However, there are practical limits of photometry associated with the ultrafine particle size range. Generally, 100 nm is considered the smallest particle diameter that measurably contributes to a photometer signal (Hinds 1999c, Gebhart 2001). Additionally, 100 nm is the lower limit of particle size detected in the device utilized in the NIOSH test protocol (Pui and Chen 2001). This limit is

imposed by a combination of background light-scatter from the fluid medium, light sensitivity limits due to the required detection range of photometers, and limits in the photometer light-sensing optics. Figure 2-2 superimposes this lower limit of detection on the test aerosol particle size distributions. From this figure, it is apparent that most of the NaCl particles and a considerable portion of the DOP particles (by size) may not contribute to the photometric concentrations used to certify respirator filtration.

2.2 SIZE-FRACTION PHOTOMETER OUTPUT

2.2.1 Model Description

To determine the contribution to light scatter available for photometer detection by size fraction, the single particle light scatter, P_λ, was modeled for each test aerosol based upon its upstream particle size distribution and physical characteristics using MiePlot version 3.5.01 (Philip Laven, Geneva Switzerland). This software allows for modeling of various user-defined aspects of optical and electromagnetic scattering by particles in accordance with Mie theory (Bohren and Huffman 1983). The modeling parameters included challenge aerosol refractive index (NaCl: 1.544, DOP 1.485), medium refractive index (1.0), light scatter angle (45^o), and incident light wavelength of 780 nm with perpendicular polarization (National 2006a,b). The model output was the relative intensity of scattered light at 45^o.

For the model, the scattering particles were assumed to be homogenous spheres. This is very nearly the case for the DOP aerosol particles, but not for the NaCl particles, which are a face-centered cubic crystal structure. Previous studies have shown that Mie scattering provides a reasonable estimate of light scatter for non-spherical particles in certain

circumstances. Perry et al. studied light scatter of aerosolized salt particles up to 1 µm in diameter and observed that light scatter in the forward direction was relatively independent of shape for particles with a size parameter up to 3 (1978). For the NIOSH sodium chloride challenge aerosol, this observation would apply to particles smaller than 745 nm.

More recent work by Chamaillard et al. compared the differences in modeled light scatter estimates for sea salt crystal aerosol particles from ~ 100 nm to 2 µm in size using Mie theory and discrete dipole approximation (DDA) (2003). Discrete dipole approximation utilizes a volume integral equation to describe the interaction of electromagnetic waves and objects and is applicable to estimating scatter from non-spherical particles. Chamaillard et al. observed little or no difference in scatter between the two models for particles smaller than 300 nm. They also reported that Mie theory underestimated particle scatter by ~ 10% for salt particles greater than 800 nm. Thus, our assumption of spherical NaCl particles seems reasonable for the purpose of this investigation and did not have essential impact on our observations.

2.2.2 Model Execution

After multiplying the modeled single-particle flux of scattered light (P_λ) with the aerosol particle size density function, the size-fractioned contribution to light scatter was obtained for each challenge aerosol. These values were then summed over the challenge aerosol particle size distributions up to one micrometer:

$$R_{cum} = \sum_{0nm}^{1000nm} f(d_p) P_\lambda(d_p) \tag{3}$$

The above cumulative scatter function was plotted with the challenge aerosol count and mass cumulative functions to provide a side-by-side comparison of cumulative count, mass, and light-scattering response for the two challenge aerosols.

2.3 RESULTS AND DISCUSSION

The results of our analysis are shown in Figures 2-3a and 2-3b. In addition, Table 2-3 presents a summary of the size-fractioned contributions to count, mass and scatter for each NIOSH challenge aerosol.

The figures allow determining the count or mass of the challenge aerosols relative to light scatter. It is seen from Figure 2-3a and Table 2-3 that NaCl particles smaller than 100 nm comprise 68% by count and about 8% by mass, but essentially do not contribute to the light scatter available for photometer detection. On the other hand, the largest 0.3% of particles by count and largest 21% by mass provide half the light scatter. Approximately 80% of the light scattering is provided by particles 270 nm and larger.

For DOP, Figure 2-3b and Table 2-3 show a similar result: ultrafine particles of DOP which comprise 10% of the count and about 0.3% of the mass have essentially no contribution to light scatter, while the largest 3% of particles by count and largest 30% by mass provide half the light scatter. Approximately 80% of the light scattering is provided by DOP particles 350 nm and larger.

The analysis indicates that the NIOSH certification test protocol (as directed by 42 CFR 84.181) effectively does not measure the contribution to filter penetration made by particles in the ultrafine size range. Particles less than 100 nm in size are present in both challenge

aerosols; however, these particles essentially do not contribute to the photometer signal used for measuring the aerosol concentration. The certification protocol—as it is now administered—has limited ability to provide users with information on the respirator filter efficiency against ultrafine particles. This finding seems to be of particular importance due to the uncertainties of health effects associated with environmental ultrafine aerosol particles and engineered nanoparticles (<100 nm). Workplace risk management of potential occupational hazards from engineered nanoparticles is an area of ongoing research. NIOSH has identified respiratory protection as a critical topic area with respect to knowledge gaps about nanotechnology and occupational health (NIOSH 2005).

The existing certification test may not assess filtration efficiency for the particle sizes that represent the "worst case scenario" in terms of collection by respirator filters with electret properties (i.e., exhibit the highest penetration). The MPPS for a specific filter system is determined by several factors, including airflow, fiber charge density, and aerosol particle charge distribution (Kanaoka et al. 1987, Stevens and Moyer 1989a, Hinds 1999a,b, Martin and Moyer 2000). The NIOSH presumption of a most penetrating size of approximately 300 nm (MMAD) may not hold for electret filters under NIOSH test conditions. A summary of evidence in support of this is shown in Table 2-4 and discussed here. It has been shown with NaCl challenge aerosol that peak aerosol particle penetration through polypropylene electret filter material may occur at particle diameters much less than 300 nm. The MPPS, which has been shown to decrease with increasing filter face velocity, appears to be consistently less than 100 nm for aerosols in uncharged and Boltzmann charged conditions. Baumgartner and Loffler (1986) evaluated two types of electret filters against 20–250 nm NaCl particles in charge equilibrium. For split-type fibrous filters, peak penetrations

occurred at approximately 30 nm while they were observed at ~ 70 – 80 nm for the electrostatically spun filter. Lathrache and Fissan (1986b) tested three types of commercially available electret filters against the Boltzmann-charge-equilibrated NaCl and diethylhexyl-sebacate oil (DES) aerosols with a particle diameter of 20 nm to 1 µm at varying face velocities. Penetration was observed to have a bimodal dependence upon particle size, with a MPPS below 100 nm in six of eight test conditions. Kanaoka et al. (1987) evaluated a rectangular fiber electret filter against NaCl aerosol particles ranging from 20 to 400 nm in various charge states. The observed MPPS of uncharged and equilibrium-charged particles was less than 100 nm. Oh et al. (2002) performed a numerical simulation of single fiber filtration efficiency of a unipolar charged fiber against particles smaller than 1 µm and compared results to laboratory measurements. Using a semi-empirical approach, the authors incorporated simulation of filter deposition by mechanical and electrical means. The model—being in agreement with experimental data—predicted a MPPS of ~ 85 nm.

In addition to testing filter materials, evaluations have been conducted specifically with respirators utilizing electret filters. Brosseau et al. (1989) evaluated the collection efficiency of 10 electret dust/mist filters against latex spheres 102 nm to 2 µm in size at a Boltzmann charge distribution. Peak penetration for all filters was observed to occur at the smallest test particle size of 102 nm; results suggested a MPPS equal to or less than 102 nm. Stevens and Moyer (1989) tested various types of air-purifying respirator filters against NaCl and DOP aerosols in Boltzmann charge distribution with a particle size ranging from 30 to 300 nm at varying face velocities. Peak penetration was observed to be below 100 nm for all filter types, except for the tested high efficiency (HE) filters—a precursor designation to the current N or P-100 type filters. Fardi and Liu (1991) evaluated several models of filtering facepiece

respirators (FFR) against charge-neutralized NaCl and DOP aerosols from 35 nm to 4 µm in size. Those respirators with electret properties showed peak penetrations at approximately 100 nm while those without were between 300 and 400 nm. More recently, Martin and Moyer (2000) studied the size-fractioned filtration efficiency of various FFR's against both NIOSH certification test aerosols before and after removing the filter electret charge. They observed that removing the electret charge resulted in significantly higher penetration and a shift in the MPPS from 50–100 nm to >250 nm. Our team at the University of Cincinnati studied the size-fractioned penetration of aerosolized NaCl particles and MS2 bacteriophage virions with a Boltzmann charge distribution through N95 filtering facepiece respirators and observed a MPPS < 100 nm (Balazy et al. 2006a,b). Richardson et al. observed similar results when testing N95 FFR's and cartridges under varying constant and cyclic flow conditions using neutralized NaCl, DOP, and MS2 bacteriophage aerosols (2006). Lastly, NIOSH researchers recently published a study of size-fractioned N95 FFR penetration using Boltzmann-charged NaCl aerosol 20 to 400 nm in size. They consistently observed a MPPS of approximately 40 nm (Rengasamy et al. 2007).

According to conventional mechanical filtration theory (Hinds 1999b), a NaCl aerosol with a physical particle diameter of ~65 nm equates to a MMAD of ~300 nm (which is the currently accepted MPPS). It is important to note that for electret filters the aerosol filtration in the ultrafine size range is governed by the physical particle diameter rather than the aerodynamic diameter. This is supported by the observation that aerosols at or near unit density, including paraffin, DOP and DES oils, polystyrene latex (PSL), and MS2 bacteriophage virus, also show a MPPS less than 100 nm for electret filters, as discussed above.

There are two primary findings of this study. First, our analysis shows limitation of the 42 CFR 84.181 respirator certification protocol as it is currently implemented, which does not assess filtration of ultrafine particles. The particles <100 nm essentially do not contribute to the light scatter available for photometer detection and those between 100 and 200 nm contribute rather little, so that the photometer-measured filtration efficiency is not determined for the above fractions. Second, based upon a review of the existing literature, the most penetrating particles for electret filters appears to belong to the ultrafine size fraction when challenged with an aerosol with a Boltzmann charge distribution. The contribution to light-scatter determined in our analysis is dominated by particles larger than 200 nm in both test aerosols (NaCl and DOP) while representing 6% of the NaCl and 43% DOP particles by count. According to Martin and Moyer (2000), quantifying filter penetration by count methods will "always be equal to or exceed a photometrically determined value." The results of this study provide one explanation for this: light-scatter is dominated by the larger particle sizes in the test aerosols, and those particles most likely to penetrate the filter are not measured by photometric means for electret filters.

The particle size fraction <200 nm, for which the limitations of the existing respirator testing protocol were demonstrated, can represent various workplace aerosols, including welding fume, diesel particles, viruses or viral droplet nuclei, bioaerosol fragments, and engineered nanoparticles. The filter certification via photometry seems to be most appropriate for the respirators used against aerosol hazards with mass-based exposure metrics. Research has suggested that as particle size decreases, particle surface area or count becomes a better predictor of health effects than aerosol mass (Oberdörster et al 2005, 2007). Using photometry for filter testing implies a greater toxicological importance to protect users from

particles with greater mass. The concept that “more mass means greater health effects” is no longer axiomatic within industrial hygiene practice. Given the wide range of occupational aerosols, it may be that no single aerosol detection method can serve all needs. Particle count may be more appropriate than photometric methods when testing respirator filters for use against certain hazards. This approach would be able to detect and enumerate particles smaller than 100–200 nm and commercial technology for this does exist. To ensure a “worst case” testing scenario—in terms of the ability to detect the most-penetrating particle size—a count-based method of aerosol detection would appear to be preferable.

Estimating ultrafine particle penetration in conjunction with the existing respirator certification protocol may be possible using one of two strategies: 1) modeling that would require using proprietary filter specification data, or 2) additional data collection to establish a reliably predictive correlation between penetration above and below 100 nm. This last strategy is promising as shown recently by Rengasamy et al. (2007). In their study, penetration of five N95 respirator filters using the NIOSH certification protocol was plotted against count-based penetration of 40 nm monodisperse particles and the relationship described using regression. They showed that 1) the relative performance of respirators was similar for particles above and below 100 nm among the respirators tested, and 2) a descriptive relationship to predict respirator performance below 100 nm using the existing NIOSH protocol data may be possible. However, significant additional study would be required to derive a reliably predictive relationship for filter cartridges and facepieces in each filter class. Consideration should be given to pursuing this approach further.

2.4 CONCLUSIONS FOR SPECIFIC AIM 1

The physical characteristics of aerosols used in the current NIOSH respirator certification/testing protocol were reviewed. According to the protocol, filtration efficiency is determined by measuring the aerosol concentrations upstream and downstream of a filter using a forward light-scattering photometer, which is capable of adequately measuring light scatter of particles significantly above 100 nm. The presently accepted protocol has limited ability to measure the contribution of smaller particles, especially in the ultrafine fraction (<100 nm). The latter include the particles which have been shown to exhibit the highest penetration through electret filters under NIOSH test protocol conditions. Additionally, the information provided by the certification test does not allow evaluating how penetration varies based upon particle size. It was concluded that while the NIOSH certification is effective at determining filtration efficiency against the majority of workplace aerosols, it is generally limited to providing respirator users performance data for particles greater than about 100 nm in physical diameter.

SPECIFIC AIM 2

Determine and compare the filter penetration and most-penetrating particle size for a selection of N99 and N95 filtering facepiece respirators when challenged with inert (NaCl) and biological aerosols (3 test viruses) which possess significant ultrafine fractions.

3.1 INTRODUCTION

The purpose of this investigation was twofold: 1) to evaluate size-fractioned filter penetration of N99 FFRs against inert and biological ultrafine aerosols at a wide range of inhalation flow rates – from 30 to 150 L/min; and 2) to compare respirator filter penetration values within and between filter classes, model, and challenge aerosol type (inert and biological). Thus, it is intended to serve as a follow-up of our previous work (Balazy et al. 2006a,b) that examined N95 respirators at 30 and 85 L/min. The data collected in the present study provide respirator users with additional information for comparing filtration of N99 and N95 FFRs against ultrafine particles, including virions.

3.2 METHODS

3.2.1 Study design

The initial filter penetration through two N99 FFRs and one N95 FFR (selected for comparison) was evaluated at three flow rates (30, 85, and 150 L/min) against two types of challenge aerosol: inert and biological. The selected inert aerosol, NaCl of ~20 – 500 nm in

particle size was utilized in testing all three respirators; the biological aerosols included MS2 bacteriophage virus (used to test all three respirators), *Bacillus subtilis* bacteriophage virus (N95 respirators), and enterobacteriophage virus type T4 (N95 respirators). Since most occupational exposures to ultrafine particles are low in mass terms and most FFR's are intended to be disposable respirators, the majority of their use (particularly in health care settings) will be in conditions with little or no particle loading. Therefore, this study examined only initial respirator filter performance and did not address filter loading. Additionally, this study did not evaluate the respirator face-seal leakage.

3.2.2 Test system

The test system presented in Figure 3-1 was described in earlier publications (Balazy et al. 2006a,b). The challenge aerosol penetration through the respirators was evaluated using a manikin-based protocol. The respirator was sealed to a manikin face, leak-tested, and placed inside of a 0.096 m^3 test chamber. The challenge aerosol concentrations were measured upstream and downstream of the respirator facepiece. The aerosols were generated with a 6-jet Collison nebulizer (BGI Inc., Waltham, MA), diluted and dried with clean air, charge-equilibrated to a Boltzmann charge distribution using a Kr^{85} sealed source (Model 3054, TSI Inc., Minneapolis, MN), and fed to the top of the test chamber. Constant inhalation flow was drawn through the probed manikin while size-fractioned particle counts from 20 to 500 nm in diameter were recorded outside and inside of the respirator facepiece using a Wide-range Particle Spectrometer (WPS, model 1000 XP, MSP Corp., Shoreview, MN) connected to the data acquisition system.

3.2.3 Respirator selection and test conditions

The two models of N99 and one model of N95 FFRs selected for this study are commonly used in industry and healthcare settings, based on the recommendations from the University of Cincinnati Occupational Pulmonary Services (Director, Dr. Roy McKay) that performs respirator fit testing and training for numerous industries in the U.S. The N95 respirator was of the same make and model as tested in our previous studies (Balazy et al. 2006a,b). This model demonstrated relatively higher filtration of ultrafine particles when compared to other N95 models evaluated in our laboratory. Different manufacturers supplied the two N99 respirators (N99-A and N99-B).

The constant airflows (Q) of 30, 85, and 150 L/min were selected to represent different inhalation regimes. The first represents inhalation during low/moderate intensity work. The second corresponds to a hard work load and is used by NIOSH for respirator filtration certification. The flowrate of 150 L/min was intended to represent an instantaneous peak inspiratory flow (PIF) during moderate to strenuous work (Cassidy et al. 2003, Lafortuna et al. 1984, Harber et al. 1984). Consensus is not found in the literature for a representative occupational ventilation rate for PIF. However, the range of PIFs for the 95^{th} percentile minute volume for occupational tasks is estimated to range between 182 and 295 L/min (Caretti et al. 2004). Therefore, the choice of 150 L/min may underestimate a "worst case" PIF. Studying respirator filtration at higher inhalation flow rates is salient, at least, for two reasons. First, the rate established by the NIOSH protocol (85 L/min) may be exceeded during more strenuous occupational tasks. Second, modern FFR media relies upon electret properties for much of the overall filtration efficiency (Martin and Moyer 2000, Caretti et al. 2004). For ultrafine particles, the primary filter capture mechanisms are diffusion and

electrostatic interaction, which are both strongly dependent upon respirator face velocity. This suggests the lowest collection efficiency (highest penetration) at the highest inhalation flow rate (Lathrace and Fissan 1986a,b, Lee and Mukund 2001).

Temperature and relative humidity were monitored during the tests using a DeltaTrak Thermo-Hygrometer (model 13306, DeltaTRAK, Inc., Pleasanton, CA). Relative humidity was maintained between 40 and 45% while temperature ranged from 23 to 26º C.

3.2.4 Selection and preparation of viruses

Three viruses were selected for use in filtration testing: enterobacteriophage types MS2 and T4, and bacteriophage *Bacillus subtilis* SP01. These were chosen for their small particle sizes, low pathogenicity, and ease of preparation and use. We intended to perform the tests with (a) the smallest virions as well as (b) larger ones – of similar dimensions to those of the SARS coronavirus [~80 nm diameter (Goldsmith et al. 2004)] and influenza A virus subtype H5N1 [~80 – 100 nm diameter (Madigan and Martinko, 2006a,b)]. MS2 has about the smallest size among viruses. T4 and *B. subtilis* bacteriophage are larger and close to the SARS coronavirus and H5N1 by their volumetric equivalent sizes. It is acknowledged, however, that the latter two simulants are considerably different from the targets in terms of virion shape and aspect ratio, which may influence their filtration properties (Willeke et al. 1996, Flagan 2001, Rengasamy et al. 2004). This is addressed further in the discussion.

MS2 is an icosahedral RNA bacteriophage which infects the male *Escherichia coli* bacteria (Valegård et al. 1990). An icosahedron is a symmetric polyhedron with 20 triangular faces (Figure 3-2); its shape is close to spherical (Madigan and Martinko, 2006a). A single MS2 virion has a physical diameter of ~ 28 nm (Valegård et al. 1990, Madigan and Martinko

2006b). T4 bacteriophage—which also infects many *Escherichia coli* bacterial strains—is a double-stranded DNA bacteriophage with asymmetric icosahedral head, helical tail, endplate and tail fibers as shown in Figure 3-2. A mature T4 virion is non-spherical. It is approximately 225 nm along its longest axis including the head (~ 85 x 100 nm), the tail (~ 25 x 100nm), and the endplate (~ 50 x 25 nm) (Leiman et al. 2003). *B. subtilis* bacteriophage SP01 is also a double-stranded DNA bacteriophage with a structure similar to that of the T4 bacteriophage except with a roughly symmetrical icosahedral head (Hemphill and Whitely 1975). A mature *B. subtilis* bacteriophage SP01 is typically 237 nm along its longest axis with a head and tail that measure 87 x 90 nm and 20 x 147 nm, respectively (Hemphill and Whitely 1975).

MS2 (ATCC 15597-B1) and *B. subtilis* bacteriophage (ATCC 27370-B1) suspensions were prepared using lysis of host bacterial solutions — *E. coli* (ATCC 15597) and *B. subtilis* (ATCC 27370), respectively. This was followed by centrifugation to remove bacterial cells, their debris and particles from the medium then filtration with 0.4 µm sterile Millipore filter (Millipore Corp, Billerica, MA). T4 bacteriophage suspensions were prepared from freeze-dried phage vial (ATCC 35060-B4) by adding 9 mL of Luria-Bertani broth followed by serial dilution. Suspensions of each phage for aerosol experiments were diluted to titre of 10^8 – 10^9 plaque-forming units per mL (PFU/mL) as determined by a modified plaque assay (ISO, 2000). ASTM reagent water purity type I ultrafiltered water was used for all suspensions (ASTM 2006).

3.2.5 Filter penetration and quality factor

Particle concentrations were measured size-selectively outside and inside the respirator filter when the inhalation flow was applied. The data were recorded over 24 size channels of the WPS' Differential Mobility Analyzer ranging from 0.021 to 0.449 µm in particle electrical mobility diameter. Size-fractioned penetration was calculated using equation (1). Another metric of filter performance determined in this study was the filter quality factor, q_f, which incorporates airflow resistance (characterized by the pressure drop, Δp, in mmH_20) and the particle penetration (P, %) (Hinds, 1999b).

$$q_f = \frac{\ln\left(1/P\right)}{\Delta p}$$

An ideal respirator filter is characterized by low penetration and low pressure drop. Pressure drop across the filter media was measured at each inhalation flowrate using a magnehelic pressure gage (Dwyer Instruments, Inc., Michigan City, IN).

3.2.6 Data analysis

The tests were replicated three times for each of the tested respirators and challenge aerosols. The mean, peak, and standard deviation of the size-fractioned particle penetration were calculated for each combination of respirator, airflow rate, and challenge aerosol. The pressure drop measured for a given respirator and airflow was applied to the corresponding size-fractioned penetration value to obtain the filter quality factor. Mean penetration (± 1 standard deviation) and filter quality factor were then plotted against electrical mobility particle diameter.

Between-respirator comparisons of the aerosol penetration were performed for two challenge aerosols: NaCl and MS2. The particle penetration through filters of all three respirator models was compared first using NaCl data and then using MS2 data. Within-

respirator comparisons of penetration values for NaCl versus MS2 were also performed for all three tested respirator models. This database allowed us to compare the filter penetration of inert NaCl particles and airborne virions of the same particle sizes. Lastly, a within-respirator comparison with respect to penetration of NaCl, *B. subtilis* bacteriophage, and T4 bacteriophage was performed for the N95 respirator. This also allowed comparing the filtration efficiency of inert particles to that of two biological aerosols. Overall, six comparative analyses were performed, as summarized below:

Between-respirator comparisons:

(1) NaCl challenge aerosol: compare penetration through N99-A, N99-B, and N95 filters;

(2) MS2 challenge aerosol: compare penetration through N99-A, N99-B, and N95 filters;

Within-respirator comparisons:

(3) Model N99-A: compare penetration of NaCl and MS2;

(4) Model N99-B: compare penetration of NaCl and MS2;

(5) Model N95: compare penetration of NaCl and MS2;

(6) Model N95: compare penetration of NaCl to that of phage *B. subtilis*, and phage T4.

Comparisons 1 and 2 were performed using analysis of variance (ANOVA). Comparisons 3–5 were run using Student's t-test (Fisher's LSD). Both ANOVA and Student's t-test with Bonferroni adjustment were utilized for Comparison 6. All tests were performed using Excel (Microsoft Corp., Redmond WA) at a significance level of 0.05.

3.3 RESULTS AND DISCUSSION

3.3.1 Aerosol penetration and filter quality factor

Aerosol penetration, pressure drop, and quality factor for each test aerosol and inhalation flow rate are summarized in Table 3-1. For NaCl, the following specific particle sizes and ranges were selected for this summary table:

(1) 0.1 µm representing the approximate mobility sizes of phage *B. subtilis*, and phage T4;

(2) 0.3 µm representing the presently accepted MPPS;

(3) 0.02 to 0.5 µm (integrated mean) representing overall penetration over the entire measured range of NaCl particle sizes; and

(4) 0.1 to 0.5 µm (integrated mean) representing the particle sizes which primarily contribute to filter efficiency determination using the NIOSH certification protocol.

For viruses, the following particle sizes were designated:

(1) 0.02 to 0.09 µm to represent the nominal virion size of MS2 and to include aggregates; the rationale for the selection of this particle size range is discussed in greater detail in Balazy et al. (2006b) (note that, resulting from slightly different WPS settings, the upper limit was modified – from 0.08 µm in Balazy et al. to 0.09 µm in this study)

(2) 0.1 µm to represent the approximate mobility sizes of phage *B. subtilis*, and phage T4. A single WPS channel with a midpoint of 0.1 µm (range 0.094 µm to 0.11 µm) was used for the larger virions because a steep drop in the challenge aerosol particle size distribution beyond 0.1 µm suggested that aggregates, if present, did not considerably contribute to the total particle count.

NaCl challenge aerosol. Particle penetration increased with increasing airflow for all three respirators (Figure 3-3) with the overall mean penetration at 150 L/min exceeding that at 30 L/min by an average factor of 7.9 (N99 model A), 7.6 (N99 model B), and 5.9 (N95). For all three respirators and inhalation airflows, the MPPS was less than 0.1 µm. Peak penetrations for N99 model A were 10.2, 5.9, and 1.3%, respectively, for the high, medium, and low flow rates; mean penetration at 85 L/min was 3.2% (for all particle sizes from 0.02 to 0.5 µm) and 1.6% (calculated specifically for particles from 0.1 to 0.5 µm). For N99 model B, peak penetrations were 6.6, 4.3, and 1.0% at Q = 150, 85, and 30 L/min , respectively. The mean penetration at 85 L/min was 2.4% for particles 0.02 to 0.5 µm and 1.7% for 0.1– 0.5 µm. For the N95 respirator filter, peak penetrations were 8.1, 4.8, and 1.4% at each respective inhalation flow rate. At Q = 85 L/min, mean penetrations 2.9% (integrated 0.02 to 0.5 µm) and 1.7% (integrated over 0.1 – 0.5 µm,). Mean penetration was significantly higher for all three respirators when taking into account ultrafine sizes as compared to those >0.1 µm. The N95 data is consistent with previous observations using N95 FFRs (Balazy et al. 2006a). It is apparent from Figure 3 that penetration was quite similar between respirators even though respirator classes differed (N95 versus N99).

Table 3-1 shows the pressure drop values across the filter for each respirator and airflow. The N95 FFR demonstrated the lowest resistance at each airflow while N99 model B possessed the highest. The pressure drop values are consistent with those reported previously for N95 FFRs and N99 filter cartridges by Martin and Moyer (2000). Although Δp differed, particle penetrations appear similar. This can be explained by the charge densities carried by the filter material. Use of electret filters (with charged fibers) allows for increased filter

efficiency without increased breathing resistance. The tested N95 filter likely possesses a higher charge density and lower packing density than the N99 respirators.

The size-fractioned filter quality factor (q_f) is also shown in Figure 3-3 as a function of the NaCl particle size and inhalation flow rate. It is not as dependent on the particle size as filter penetration. While q_f is similar between respirators operating at 85 and 150 L/min, the N95 demonstrates higher quality factor at 30 L/min due to the its lower pressure drop: 2.7±0.10 mm H_20 as compared to 3.9±0.20 and 4.5±0.15 mm H_20 measured for N99-A and -B respectively. The q_f value determined for specific particle sizes of 0.1 and 0.3 µm were similar to the mean value obtained for the size range of 0.02 to 0.5 µm. Filter quality factor was significantly lower for all particle sizes (integrated mean, 0.02 to 0.5 µm) than for particles calculated specifically for >0.1 µm.

The utility of filter quality factor in assessing the respirator filter performance is not presently established. One reason is that respirator performance also depends upon face-seal leakage, which is not accounted for in filtration studies. Whether face-seal leakage and filter resistance are related in FFRs has not been thoroughly investigated. Although wearer comfort is expected to increase with increasing q_f for specific filtration efficiency, this has not been quantitatively studied, and physiologically meaningful differences of filter quality factor have not been assessed. Quality factor has been used previously as a tool for comparing respirators. Han (2000) ranked respirator performance using q_f at inhalation flow rates from 10 to 85 L/min and utilized a plot of flow rate versus q_f to compare FFR. Also, Chen et al. (1992) utilized q_f to compare performance of filtering-facepieces and respirator cartridges.

MS2 phage challenge aerosol. Table 3-1 and Figure 3-4 present the mean penetration values for MS2 virus with a designated particle size range of 0.02–0.09 µm. Populated by

single virions as well as virus aggregates, this size range accounted for ~82 % of the upstream particle count. Airflow had a strong effect: mean penetration at 150 L/min exceeded that at 30 L/min by a factor of 5.3 (N99-A), 5.9 (N99-B), and 3.3 (N95). Similarly to the trend observed with the NaCl aerosol in this study [and the conclusion made by Balazy et al. (2006a,b) for N95 FFR], At Q = 85 L/min the MPPS was < 0.1 µm for all three respirators; peak penetrations were 4.3% (N99-A), 4.6% (N99-B), and 4.3% (N95, data from Balazy et al. 2006b), while mean penetrations were 3.4, 3.3, and 3.5%, respectively. Figure 3-4 demonstrates relatively high variability in the penetration of the N95 respirator at Q = 150 L/min, but – again – the trend is consistent with previous observations at 30 and 85 L/min (Balazy et al. 2006b).

B. subtilis and T4 phage challenge aerosols. Table 3-1 and Figure 3-5 present the data for N95 respirator filter challenged with the *B. subtilis* phage and T4 phage viruses. The effect of airflow on penetration is readily apparent with the overall mean penetration at 150 L/min exceeding that at 30 L/min by an average factor of 6.6 (*B. subtilis* phage) and 9.5 (T4 phage). At 85 L/min, peak penetrations of 3.4% for the *B. subtilis* phage aerosol and 2.6% for the T4 phage aerosol occurred at 0.04 µm, which is smaller than the mobility sizes of single virions of *B. subtilis* and T4 phages estimated based on their physical dimensions. This is attributed to the presence of remnant solutes, biological fragments, and impurities associated with preparation and freeze-drying. Penetration at the single virion mobility diameter, calculated specifically at 0.1 µm and Q = 85 L/min were 1.9% (*B. subtilis* phage) and 0.95% (T4 phage). Low particle counts for the T4 challenge aerosol resulted in large standard deviations in penetration measurements above ~0.12 µm.

3.3.2 Between and Within-Respirator Comparisons

The penetration of NaCl aerosol in two particle size ranges was compared between respirators as shown in Figure 3-6. Although we expected differences in filtration between respirator classes (N99 was expected to be more efficient in collecting particles than N95), no significant differences in mean penetration were observed for the range of 0.02–0.5 µm (Fig. 6a) or 0.1–0.5 µm (Fig. 6b). However, due to the small sample size, we fall short of concluding that the performance of N99 FFRs is generally no better than that of N95 FFR for the particles up to 0.5 µm. It seems more reasonable to state that filtration of a "better" N95 FFR may approach the performance of some N99 FFR models over the particle sizes observed here when measured by count.

Mean penetration was also compared by particle size range and differed significantly; when analysis was limited to particles of >0.1 µm, mean penetration for all three respirators was significantly lower ($p = 0.01$) than for particles ranging from 0.02 to 0.5 µm. The greatest contribution to penetration occurred at <0.1 µm for all three respirators. Utilizing a protocol that can also measure the ultrafine component of the test aerosol may result in discovering significantly higher filter penetration (by particle count) than it is anticipated. These observations do not mean that the tested respirators fail to comply with their respective NIOSH certification criteria because the NIOSH certification protocol uses a different method to measure aerosol concentrations to calculate filter penetration (DHHS 1995).

While no differences were observed between respirators when comparing mean penetration of MS2 aerosol in the designated particle range of 0.02 to 0.09 µm (see Figure 3-7), we also compared the penetration of NaCl to (1) the MS2-containing aerosol for each respirator over the integrated size range of 0.02 to 0.09 µm (Figure 3-8); and (2) the two

larger phages *B. subtilis* and T4 at their estimated mobility diameter of 0.1 µm (see Figure 3-9). The Figures show comparisons for Q = 85 L/min. These served as direct comparisons of inert particle penetration to that of aerosols containing biological particles over the same mobility diameters. Two differences were observed. At 150 L/min, there was a significant difference in penetration between MS2 (5.4%) and NaCl (8.5%) for N99 model A over the integrated size range of 0.02 to 0.09 µm (p=0.01, not shown in figure). Also, we found a significant difference between NaCl and T4 phage at 85 L/min (p = 0.005) where T4 phage penetration was 0.95% compared to 2.6% for NaCl (Figure 9) for the N95 FFR. Overall, no biological aerosol penetration exceeded that of inert aerosols.

Several properties of airborne virus particles may have influenced filtration in this study and could have contributed to the observed—although inconsistent—differences between the inert and biological aerosols. Particle parameters that effect diffusion, the electrostatic collection mechanism, or particle adhesion to the filter fibers are believed to be relevant. Particle shape may affect virus particle filtration since it can influence its polarization and formation of dipole charges in an electrical field (Flagan 2001). Also, shape can influence particle drag by altering terminal velocity toward an influencing fiber, changing the probability of capture (Flagan 2001). Dynamic shape factors that aid in describing behavior of airborne virus particles have not been investigated. Lastly, shape may also influence filtration through particle rebound. Boskovic *et al*. (2005 and 2007) recently observed differences in filtration efficiency between spheres and perfect cubes of the same electrical mobility diameter up to 0.3 µm. Greater penetration of cubes was ascribed to differences in rebound probability during tumbling at the fiber surface. It is not presently known how the shape of a virus aerosol particle may affect its rebound during filtration.

Electrical properties of virions may also influence filtration. With a neutralized aerosol, the virus particle permittivity or dielectric constant is of interest. This represents the ability of a particle to polarize when in an electric field. The degree of polarization will be proportional to the force of attraction between the particle and the influencing fiber (the polarization force). It has been shown theoretically and experimentally that particles with high dielectric constant are captured by an electret filter with greater efficiency than those with low dielectric constant (Oh et al. 2002, Yang and Lee 2005, Wei et al. 2006). The dielectric constant of NaCl is ~6. While the dielectric constant of the tested virions is not known, similar-size virions have been estimated to have dielectric constants of greater than 55 (Aristides et al. 2007, Lepizco-Encinas and Rito-Palomares 2007).

3.4 CONCLUSIONS FOR SPECIFIC AIM 2

The penetration of four challenge aerosols through three N-type filtering-facepiece respirators at three inhalation flow rates was determined. Challenges remain in aerosolizing viruses with the intention of creating a monodisperse aerosol consisting of single virions. As seen in this and other studies, remnant solutes, biological fragments, and the possibility of aggregate formation can significantly contribute to the resulting particle size distributions.

Inhalation airflow had a significant impact upon particle penetration. The primary mechanisms of ultrafine particle capture – diffusion and electret charge interaction – are heavily influenced by the filter face velocity. Since the selected 150 L/min flow may underestimate the 95^{th} percentile peak inspiratory flow (PIF) during occupational tasks, additional study seems feasible in this area to better define a very severe or worst case

condition. Also, further study of respirator penetration during cyclic breathing with high PIF's is needed.

The pressure drop across the filters was determined and the filter quality factor calculated providing information on relative performance of the respirator filters. However, the salience of this information without reference to performance during respirator wear is limited. Investigation of whether filter quality factor is predictive of actual workplace protection would determine whether it is a meaningful metric of FFR performance.

The MPPS was <0.1 µm for all aerosol challenges. This has been demonstrated previously for electret-type filter materials using physiologically relevant airflows. As a corollary, we also observed that overall respirator penetration increases significantly — when measured by count — if the ultrafine fraction of the test aerosol is properly detected and included in the integration. This finding is important because the NIOSH filter certification protocol assumes a most-penetrating particle size of 0.3 µm (by mass) and cannot adequately measure aerosol particles below 0.1 µm due to limitations of photometry.

We observed that a better performing N95 FFR can approach the filtration performance of some N99 FFRs over the tested particle size range. However, this should be considered with caution and not generalized because the presented results were obtained for a single model of N95 compared to two specific models of N99.

Overall, viral penetration through the tested FFRs did not exceed that of inert NaCl aerosol. We observed a difference between inert and bioaerosol filtration where NaCl penetration exceeded that of MS2 (for N99 model A at 150 L/min) and that of T4 phage (for N95 FFR at 85 L/min) which may be attributed to a number of causes. The results suggest

that inert aerosols may generally be appropriate for modeling filter penetration of similarly-size viruses.

SPECIFIC AIM 3

Develop a method to determine and compare the physical (in terms of particle count) and biological (in terms of viable count) filtration efficiency of two traditional respirators and one novel design of a filtering facepiece respirator using an iodinated treatment of the filter structure.

4.1 INTRODUCTION

The purpose of this study was to develop and assess the feasibility of a test protocol that integrates common instruments and approaches and enables the above-mentioned differentiation when respirator filters are challenged with viable bioaerosol particles, including single virions representing the most penetrating particle size for electret filters.

To fully assess the total particle filtration efficiency and antimicrobial effect of a bioaerosol-filter interaction, an ideal protocol should differentiate between physical (often referred to as "total") filtration efficiency and viable filtration efficiency. Physical filtration efficiency ($\eta_{physical}$) is defined as the percentage of incoming particles/microorganisms that have been collected by the filter, regardless of their viability. Viable filtration efficiency (η_{viable}) is usually derived using culture-based analysis and is the percentage of culturable microorganisms collected by the filter. For a respiratory protective device with no antimicrobial capability, $\eta_{physical}$ and η_{viable} should be the same. For a filter to exhibit biocidal effect, η_{viable} must exceed $\eta_{physical}$. In the latter case, both the physical and viable efficiencies should be known. Figure 4-1 schematically represents a notional example using an

antimicrobial filter challenged with bioaerosol particles some of which are viable. As seen from this example, $\eta_{physical}$ is

$$\eta_{physical} = \left(1 - \frac{3}{10}\right) \times 100 = 70\%$$

and η_{viable} is

$$\eta_{viable} = \left(1 - \frac{1}{9}\right) \times 100 = 88\%$$

.

The 18% difference between the collection efficiencies of total and viable bioaerosols occurring due to the respirator antimicrobial properties translates into a 2.5-fold difference (30% versus 12%) in bioaerosol penetration through the filter media (P = 100% – η).

4.2 MATERIALS AND METHODS

The test respirators were challenged with viable aerosol of MS2 bacteriophage (ATCC, 15597-B1) obtained from the American Type Culture Collection (ATCC, Rockville, MD), and the physical and viable filter penetrations were determined simultaneously. The experimental facility is schematically shown in Figure 4-2. Physical filter penetrations were measured using a manikin-based protocol described in the previous chapters. The particle penetration rather than collection/filtration efficiency seems to be a convenient measure to compare performance of highly efficient filters (with η close to 100%); therefore, it was used for the data presentation in this study.

MS2 challenge aerosol was generated with a 6-jet Collison nebulizer (BGI Inc., Waltham, MA). Dried with clean air and charge-equilibrated to a Boltzmann charge distribution with a Kr^{85} sealed source (Model 3054, TSI Inc., Minneapolis, MN), the

challenge bioaerosol entered a 0.096 m^3 test chamber that housed a manikin with the tested respirator sealed on it. A constant volumetric flow of 85 L/min was drawn for 15 min through the probed manikin. Size fractioned concentrations of aerosolized MS2 bacteriophage were measured outside ("upstream") and inside ("downstream") of the respirator using a Wide-range Particle Spectrometer (WPS, model 1000 XP, MSP Corp., Shoreview, MN). The lower particle size limit of the WPS is 10 nm. Since its first five measurement channels (10 – 17 nm) recorded too few particles (<20 per channel) inside the respirator and represented sizes considerably below the size of MS2 virions, the data were plotted starting from 17 nm. Extending the particle size scale up to 100 nm enabled us to include the nominal MS2 virion size as well as most-penetrating particle size range known for respirators utilizing electret filter media when challenged with neutral particles. The aerosol chamber was enclosed in a Biosafety Level II cabinet (SterilchemGARD, Baker Co., Sanford, ME).

Respirator leakage was not assessed as the respirators were sealed to manikin faces and leak-tested. Also, since we studied the initial filtration, the study did not aim at quantifying the change in the filter efficiency over time due to particle loading.

Physical filter penetration was calculated as a ratio of the downstream, C_{down}, and upstream, C_{up}, concentrations and plotted as a function of the particle size:

$$P_{physical} = \frac{C_{down}}{C_{up}} \times 100\% .$$

Bacteriophage MS2 was used as the challenge aerosol because of its small particle size— ~ 28 nm in physical diameter (Valegård et al. 1990, Madigan and Martinko 2006b)— ease of preparation, low pathogenicity, and history of utilization as a simulant of pathogenic viruses (Shin and Sobsey 2003, Tsend and Li 2005). Methods to prepare the MS2

bacteriophage for aerosolization have been described previously. MS2 aerosol suspensions had a typical phage titer of 10^9 plaque-forming units of MS2 per milliliter of solution (PFU/mL) determined using the modified plaque assay (ISO 2000).

In parallel to the real-time measurement, MS2 bacteriophages were collected outside and inside the respirator using 25 mm gelatin filters with a 3 µm pore size (Sartorius AG, Göttingen, Germany obtained through SKC, Inc.) within sterilized 25 mm filter holders (SKC, Inc., Eighty Four, PA) at a flow rate of 4 liters per minute, calibrated pre and post-sampling. This method has shown good collection efficiency (> 93%) and maintenance of MS2 viability (Jaschhof 1992, Burton et al. 2007, Grinshpun et al. 2007). Gelatin filters were then dissolved in sterile filtered water and mixed (Touch mixer-Fisher Scientific Inc.). Aliquots of dissolved gelatin filter extract were serially diluted and used for plaque assay to determine the number of airborne culturable MS2 virions (PFU per cm^3 of air sampled) using *Escherichia coli* (ATCC 15597, strain C3000) as the host organism.

The viable filter penetration was determined as the ratio of concentrations of culturable viruses downstream ($C_{V\,down}$) and upstream ($C_{V\,up}$), respectively:

$$P_{viable} = \frac{C_{V\,down}}{C_{V\,up}} \times 100\% \quad .$$

Based on the WPS measurements, the electrical mobility diameter of 22 to 29 nm was designated for MS2 virions. This particle size range was selected to represent single MS2 bacteriophage virions, which have been observed to have an electrical mobility diameter of approximately 24 nm and because it matched discrete channel particle size boundaries used by the WPS (Hogan et al. 2006). The mean penetration for particle sizes integrated from 22

to 29 nm in electrical mobility diameter was calculated to obtain the physical penetration in the size range of viral particles ($P_{physical\text{-}virus}$).

Two commercially available conventional N95 filtering-facepiece respirators (FFR), produced by different manufacturers, and one commercially available iodinated polymer P95 FFR utilizing a filter treated with iodinated resin powder (10 g/m^2) were tested with respect to their filtration of viruses - total and viable. The mean and standard deviation for P_{viable} was calculated for each respirator and compared to the $P_{physical\text{-}virus}$ using t-test (if normal) or the Mann-Whitney test (if non-normal). The conventional respirators served as controls to validate the test method and were expected to demonstrate that P_{viable} and $P_{physical\text{-}virus}$ did not differ significantly.

Additionally, swatch tests were performed primarily to investigate if the presence of the antimicrobial additive influenced the media's physical filtration characteristics. Two filter swatches were specially manufactured utilizing the tested P95 filter material: one with no iodinated resin added and one with a 3.5-fold greater amount of the powder per unit surface area (35 g/m^2) as compared to the commercially available FFR. These filter swatches (as well as the swatch of the commercial P95 with a filter material that had undergone "normal" treatment) were tested following a modified protocol, in which they were mounted inside 47 mm stainless steel filter holders (model 2220, Gelman Life Sciences, Ann Arbor, MI)—as an alternative of sealing them on the manikin face—and challenged with a NaCl aerosol.

Five tests were performed for each commercial respirator model (two control N95's and one P95) as well as for specially manufactured filter swatches. The respirator tests used a constant airflow of 85 L/min—the same used by NIOSH in their certification protocol. The

swatch tests were conducted at a much lower flow rate (~3.9 L/min) to achieve the same face velocity as that in the respirator test (~7 cm/s).

When any additive is incorporated in the respirator filter material, especially of potential biocidal properties, it is important to identify whether this additive may be released (as a gas or aerosol) during breathing and inhaled by a wearer. To quantify the iodine release from the iodine-treated respirator filter, a separate experiment was designed. A swatch of filter material with the higher amount of iodine powder (35 g/m^2) was exposed to a constant air flow and the overall iodine release (in mg) was measured as a function of time. The test system consisted of a vacuum pump and timer as well as the necessary tubing and flow meters capable of measuring the required flows. A flow rate of 42.5 L/min was established through a 100 cm^2 filter area over an 8-hour period. The iodide released was measured using an HPLC (model DX600, Dionex Corporation, CA). The main components of this instrument were an auto sampler, chromatography oven, pulsed electrochemical detector containing a silver working electrode and silver/silver chloride reference electrode, gradient pump and compressed helium gas tank with regulator. The quantity of iodine was initially measured in parts per million and then transformed into an iodine concentration (mg/m^3). The measured cumulative mass of the iodine downstream of the filter represented a conservative scenario aiming at simulating (a) a moderately to hard work breathing during a full work shift and (b) excessively powerful source of iodine that can potentially be released. The instrument's limit of detection was 1.6×10^{-3} mg/m^3. The measurements were conducted in six replicates.

4.3 RESULTS

In Figure 4-3, the size-fractioned physical penetration of the challenge aerosol (measured from 17 to 100 nm) is shown superimposed by the viable penetration in the virus-designated mobility-based diameter of 22 – 29 nm. Each area represents the mean penetration plus and minus one standard deviation. As expected, no statistically significant differences between P_{viable} and $P_{physical\text{-}virus}$ were observed for the two control respirators calculated as integrated means over the particle sizes from 22 to 29 nm ($p>0.05$). Additionally, no significant difference was observed between P_{viable} and $P_{physical\text{-}virus}$ for the iodinated P95 respirator. The first control N95 respirator demonstrated a $P_{physical\text{-}virus}$ of 1.5±0.26% (with a coefficient of variation, CV, of 0.17) and a P_{viable} of 1.8±0.83% (CV = 0.46) (Figure 4-3a). Physical penetration for the second control N95 respirator, shown in Figure 4-3b, was 1.82±0.37% (CV = 0.20) and viable penetration was 1.7±0.78% (CV = 0.46). As seen from Figure 4-3c, the iodinated polymer respirator demonstrated very efficient overall filtration with a physical penetration of 0.012±0.006% (CV = 0.5) and viable penetration of 0.016±0.001% (CV = 0.06).

Because initial testing of the P95 respirator demonstrated higher filtration than expected for a class 95 FFR, additional filter swatch testing was performed to investigate if the iodinated powder influenced filter behavior. The test results obtained with the iodinated polymer P95 filter swatches of different powder loads challenged with NaCl aerosol are shown in Figure 4-4 as best-fit polynomial regressions of mean penetration. The size-fractioned penetration curves are presented respectively for the filter swatches with (a) no iodinated resin powder treatment, (b) normal iodine resin powder load of 10 g/m^2 used in the

commercially available unit, and (c) high load of 35 g/m^2. The mean penetration integrated for the MS2 particle size range (22 – 29 nm) as well as for the entire size range of interest (17 – 100 nm) differed significantly ($p<0.01$) between the untreated and treated filter swatches ($P_{untreated} > P_{treated}$).

The iodine release by the constant inhalation air flow, detected downstream of the filter treated with 35 g/m^2, produced a time-weighted average (TWA) air concentration of 0.04 mg/m^3. This is approximately 4% of the applicable occupational exposure limit for iodine as defined by the American Conference of Governmental Industrial Hygienists (ACGIH) and Occupational Safety and Health Administration (OSHA) (ACGIH 2003, DOL 2006). Iodine release ranged from ~ 3 µg/m^3 in the first hour and peaked at a plateau of ~ 0.1 mg/m^3 at between 6 and 8 hours of testing.

4.4 DISCUSSION

The observed size-fractioned physical penetration of challenge particles through the N95 respirator filters was consistent with previous studies (Martin and Moyer 2000, Richardson et al. 2006, Balazy et al. 2006a,b, Rengasamy et al. 2007, Eninger et al. 2008b). The filtration efficiency of the P95 respirator was greater than expected for a class 95 filter, providing filtration nearing that of a class "100" respirator filter or HEPA filter, which are limited to a penetration of 0.03% by mass of a challenge aerosol (DHHS 1995). However, it is critically important to emphasize that the purpose of this study was *not* to compare penetration between the selected respirators—this would be an inappropriate and invalid comparison because they are two different filter types—but to evaluate and compare $P_{physical-}$

$_{virus}$ and P_{viable} for a given respirator. To properly place the P95 filtration efficiency in context, it must be compared to other P95 respirators under similar test conditions. We performed an inspection of a cross-section of the P95 filter and observed that the number of filter layers and total thickness were similar to class 99 and 100 respirator filters we have tested in our laboratory. The physical properties of the filter were consistent with its high filtration efficiency.

The difference in filtration efficiencies was hypothesized to be, at least partially, attributed to the iodine resin powder, which was intended to enhance microbial filtration efficiency. The comparison of the NaCl filtration efficiencies of powder-treated and untreated P95 filter swatches enabled us to test this hypothesis. Figure 4-4 shows a trend of decreasing particle penetration with increasing loading of iodinated resin powder in the filter media. Particle penetration differed significantly between the untreated and treated filter swatches. We hypothesize that the addition of the powder increased the tortuous path length of particles as they passed through the filter medium and possibly enhanced the electrostatic interaction, thus increasing particle removal associated with the diffusion and polarization mechanisms.

The difference between the viral particle filtration efficiencies determined for the total and culturable counts was not statistically significant. This finding was anticipated for the control N95 FFRs, confirmed by the protocol, and served as a validation for the proposed test method. At the same time, we failed to observe $P_{viable} < P_{physical\text{-}virus}$ for the iodinated polymer respirator suggesting that a microbial inactivation effect was of insufficient magnitude to be detected or was not present for viral particles that penetrated the filter. It is acknowledged

that the iodine-based additive may cause an inactivation of viable viruses captured on the filter over time. However such a "long-term" effect was beyond the scope of this study.

There were notable differences in the variability of P_{viable} among the N95 respirators compared to that among the iodinated polymer P95 respirator (~7-fold in the coefficient of variation). This was likely due to the much higher upstream MS2 bioaerosol concentration and longer sample time used for the P95 FFR. To achieve a sufficiently low limit of detection for P_{viable}, two Collison nebulizers were used simultaneously to generate MS2 aerosol when testing the P95 FFR. This likely resulted in much greater precision for P_{viable}.

As indicated previously, the time-weighted average concentration of the iodine released from the P95 filters having 35 g/m^2 of iodine was considerably lower than applicable occupational exposure limits. The TWA concentration over eight hours of testing was 4% of the OSHA PEL and ACGIH TLV for iodine. The maximum iodine concentration measured occurred between 6 and 8 hours of testing and was ~ 0.1 mg/m^3. Testing for time intervals longer than 8 hours would be appropriate, although it is acknowledged this test assessed a filter with 3.5 times the iodine loading of the commercially available P95 respirator. Because widely differing approaches are being used to impart antimicrobial properties in respirator filter media, protocols to assess the user safety may differ from one product to another. This may be a consideration in future respirator certification or consensus standard requirements.

The proposed protocol appears feasible as a method to assess and potentially differentiate $P_{physical}$ and P_{viable}. There are several advantages to this protocol. Collison nebulizer—the selected method of microbe aerosolization—is inexpensive, has been used in numerous bioaerosol studies, and is capable of producing an aerosol with an ultrafine particle size fraction. Processing of the gelatin filter media for culturing MS2 virions is less labor-

intensive than other filter media which may require extensive ultrasonication. Also, measurements for $P_{physical}$ and P_{viable} take place simultaneously, which is a superior study design as compared to sequential measurements.

At the same time, the selection of the Collison nebulizer leads to some limitations of the testing protocol. First, it has been reported that the Collison nebulizer produces a time varying particle size distribution (Hogan et al. 2005), although we did not observe this during the relatively short sample periods used in this study. Second, this aerosolization method may produce MS2 aggregates that can bias the viable filtration measurement (Hogan et al. 2004). Third, contaminants from MS2 bioaerosol preparation and residual solutes aerosolized by a nebulizer may comprise a significant portion of the dried aerosol count thus masking single virions. Therefore, calculations made of $P_{physical}$ may have been influenced by particles other than MS2 virions. To examine whether this occurred, an additional study was conducted and is addressed in the following chapter using a promising aerosolization method which has recently been tested with MS2 bacteriophages to generate an "ultraclean" aerosol comprised solely of virions (Hogan et al. 2006). Overall, improvement or better validation of the aerosolization methodology can considerably enhance the filter testing protocol and provide a more definitive assessment of filtration for a bioaerosol in the ultrafine particle range.

The importance of the upstream bioaerosol concentration as a parameter that influences the utility of the proposed protocol was demonstrated. Too few bioaerosol challenge particles will result in less precise measurements for $C_{v\ down}$, and larger variance in P_{viable} values. Imprecision in either penetration measurement may mask or limit the ability of the protocol to detect differences in $P_{physical\text{-}virus}$ and P_{viable}. This is particularly relevant if testing a highly efficient filter (Wang 2004). Considering the low dose required for infection of certain

biological agents, the ability to distinguish even small differences between $P_{physical\text{-}virus}$ and P_{viable} is desirable. Protocol parameters that would influence the ability to discriminate $P_{physical\text{-}virus}$ and P_{viable} include the upstream biological particle count, sampling time, number of respirators tested, and the degree to which bioaerosols are masked by contaminants and dried solutes in a nebulizer-generated aerosol. This last factor is potentially the primary limitation of aerosolizing bioaerosols with the Collison nebulizer for the purpose of filter testing.

4.5 CONCLUSIONS FOR SPECIFIC AIM 3

The present study investigated the feasibility of a respirator filter testing protocol that enables differentiating between the physical and viable filtration when challenging respirator filters with bioaerosols. We evaluated three respirator models (two conventional N95 FFRs used as controls and one specially treated iodinated polymer P95 FFR) with aerosolized MS2 bacteriophage, and no statistically significant difference was found between $P_{physical}$ and P_{viable} for any model. The treated P95 filter efficiency was greater than expected for a class 95 respirator which was in part attributed to the iodinated resin powder, which apparently improves the filter collection, by enhancing diffusional and electrostatic polarization effects. The physical properties of the P95 filter were more consistent with a class 99 or 100 filter in terms of thickness and number of filter layers. The release of iodine vapor powder from the iodinated polymer respirator filter during inhalation appeared to be well below applicable occupational exposure limits (such as US OSHA's applicable OEL of 1.036 mg/m^3).

The protocol presented in this paper provides a tool for evaluating respirators designed to protect against bioaerosols, both viable and non-viable. At the same time, further modification or evaluation of the aerosolization system may be warranted because the aerosol nebulized from a viral suspension contains a poorly defined fraction of single viruses and is characterized by a rather broad particle size distribution. This deficiency may affect precision of the filtration measurements. Electrospray ionization shows promise as an alternative means to aerosolize viruses and create a well-characterized, monodisperse challenge bioaerosol suitable for filter testing.

COROLLARY TO: SPECIFIC AIM 3

Evaluate the efficacy and appropriateness of using nebulization for aerosolizing ultrafine MS2 for use in filter testing.

5.1 INTRODUCTION

The purpose of this study was to investigate and compare practical aspects of aerosolizing MS2 bacteriophage via nebulization and charge-reduced electrospray for filter testing applications. Each aerosolization method was evaluated and compared in the following areas: (1) viral suspension preparation protocols; (2) resulting aerosol concentration, particle size distribution and stability over short time periods (15 minutes); (3) viability; and (4) filtration behavior.

5.2 METHODS

Preparation of MS2 viral suspensions for nebulization (Balazy et al. 2006) and electrospray (Hogan et al. 2006) are described elsewhere and are briefly summarized here. The nebulizer suspensions were prepared by adding 9 mL Luria-Bertani broth with ultrafiltered water (ASTM reagent water purity type I; ASTM, 2006) to freeze-dried phage vial (ATCC 15597-B1) obtained from the American Type Culture Collection (ATCC, Rockville, MD) and serially diluted to a typical titer of 10^9 plaque-forming units of MS2 per milliliter of solution (PFU/mL) determined with a modified plaque assay (ISO 2000).

The electrospray suspension utilized MS2 bacteriophage propagated in bacterial host *Escherichia coli* (ATCC 15597) in a glucose and thiamine minimal media to a titer of 10^{10} PFU/ml. The suspension was then ultracentrifuged (30 minutes at 9000 rpm) and filtered (0.22 µm pore membrane, Fisher Scientific). Prior to electrospray aerosolization, the phage solution was again ultracentrifuged (RCF_{max} = 193000g for 6 hours) then resuspended in 10 mM ammonium acetate (Sigma-Aldrich).

Virion test aerosols were generated using a 6-jet Collison nebulizer (BGI, Inc., Waltham, MA) and an electrospray aerosol generator (model 3480, TSI, Inc., Minneapolis, MN). The nebulized aerosol was diluted and dried with clean air and charge-equilibrated using a Kr^{85} sealed source (Model 3054, TSI Inc., Minneapolis, MN); electrosprayed aerosol was dried and charge-equilibrated using a Po-210 sealed source (model 348002, TSI Inc.). Particle size distribution and size-fractioned particle counts were measured using the differential mobility analysis functions of a Wide-range Particle Spectrometer (WPS, model 1000 XP, MSP Corp., Shoreview, MN).

Short duration (15 minute) filter testing on sample filter media was performed using nebulized and electrosprayed MS2 aerosol (Figure 5-1). Tests used a swatch from the innermost filter layer of three commercially available protective masks: a commercially-available surgical mask, an N95 filtering-facepiece respirator (FFR), and an N100 FFR. Results were used to compare the MS2 aerosol filtration behavior for the two particle generation methods. Five replicates of each filter sample were tested.

Nebulizer filter test protocol. MS2 suspension was aerosolized, diluted and dried, then charge-equilibrated and passed to a 0.096 m^3 mixing chamber (Figure 5-1a). The WPS alternated samples between two identical 47 mm stainless steel filter holders (model 2220,

Gelman Life Sciences, Ann Arbor, MI): one with filter media (the "downstream" sample) and one without (the "upstream" sample). Eight samples were drawn at 1 L/min during the 15 minute filter test protocol—four each upstream and downstream. The filter face velocity was 1.7 cm/sec. Based upon the observed mobility diameter of the single MS2 virion, cumulative particle counts were recorded for mobility diameters from 23 to 26 nm. Bias between upstream and downstream sample inlets were checked for each tested filter replicate. The mean upstream and downstream particle counts, corrected for any bias, were then used to calculate filter penetration (P, in %):

$$P = \frac{C_{down}}{C_{up}} \times 100\% .$$

Electrospray filter test protocol. The MS2 solution was electrosprayed, dried, and charge-neutralized using an internally-mounted Po-210 source (Figure 5-1b). Due to the relatively low flowrate of the electrospray aerosol generator ($\leq$ 2 L/min compared to > 85 L/min for the nebulizer protocol), the aerosol flow was split and passed directly to the matched filter holders. The remaining steps matched those used for the nebulizer protocol. We also performed one round of filter testing with electrosprayed dextrose particles of the same mobility diameter as MS2 bacteriophage. This allowed a cursory comparison of MS2 filtration to that of an inert electrosprayed aerosol.

The characteristics of each test aerosol were recorded before and during each filter test. This included the particle size distribution, particle count at the nominal virion diameter, and the stability of the particle count over short durations (15 minutes). The stability of the particle count was evaluated in terms of (1) the variability in particle concentration and (2) any trends in particle count over repeated 15 minute filter tests.

MS2 Viability test protocol. Each generation method was evaluated for its ability to aerosolize viable MS2 bacteriophages with the stock suspensions from the filter tests using a method described in Chapter 4 (Jaschhof 1992; Burton et al. 2007; Grinshpun et al. 2007). Aerosolized MS2 bacteriophages were collected using 3 µm pore size 25 mm gelatin filters (Sartorius AG, Göttingen, Germany obtained through SKC, Inc.) inside sterilized 25 mm filter holders (SKC, Inc., Eighty Four, PA) at a flow rate of 4 L/min for 15 minutes. Gelatin filters were dissolved in sterile filtered water and then mixed (Touch mixer-Fisher Scientific Inc.). Aliquots of gelatin filter extract were diluted and used for plaque assay to determine the number of airborne culturable MS2 virions (PFU per cm^3 of air sampled) using host *Escherichia coli* (ATCC 15597, strain C3000). The electrospray was tested with two sheath air supplies: dry, filtered air and CO_2. Since CO_2 is better than air at preventing corona formation at the electrospray capillary tip, we wanted to observe the difference this would have on aerosolizing culturable MS2 virions.

5.3 RESULTS AND DISCUSSION

The virus preparation protocols were similar for both aerosol generation methods, with several additional concentration and purification steps applied to obtain a high-titer virus sample in aqueous ammonium acetate for electrospray aerosolization. A notable difference in the two protocols was the use of ATCC-supplied freeze-dried bacteriophage for the nebulized aerosol compared to propagating "in-house" the bacteriophage for electrospray suspension. Propagation resulted in a greater volume (liters) and higher titer (~ 10^{10} PFU/ml) at a lower cost than purchasing stock freeze-dried phage MS2. Propagating the MS2 however, required

several man-days of labor compared to several man-hours to prepare the stock MS2. Several studies have used both methods interchangeably for nebulization with no notable differences in culturability, though this observation is anecdotal as this question was not rigorously investigated (Balazy et al. 2006a, Eninger et al. 2008b). Propagating bacteriophage is more resource-intensive than using stock supplies, though anecdotally a suspension with fewer contaminants can be obtained—a desirable trait for both aerosolization methods.

Representative MS2 aerosol particle size distributions for each generation method are shown up to 100 nm in Figure 5-2. The nebulized MS2 aerosol was polydisperse, with a peak at 49 nm, geometric mean diameter (d_g) of 60 nm, geometric standard deviation (σ_g) of > 1.9, and extended out to beyond 400 nm. The distribution is similar in shape, though with a peak at a higher particle size than was observed previously where a nebulized MS2 aerosol possessed a typical peak particle count at approximately 30 (Balazy et al. 2006a). In later work in our labs at the University of Cincinnati, we have observed that a nebulized MS2 aerosol peak up to 50 nm, as observed here, is not unusual and likely has to do with the solute and contaminant content which may vary slightly from one suspension preparation to another. The 30 nm peak observed by Balazy et al (2006a) was at the time attributed to the presence of MS2 virion particles because the peak reasonably agreed with the physical size of the MS2 virion in the literature. However, it was shown that the presence of MS2 at a titer similar to that used here has little effect on the particle size distribution of an atomized aerosol (Hogan et al. 2004). Hogan's observation is corroborated in this study because the nebulized aerosol peak was typically at a larger size than the nominal virion diameter. We can reasonably conclude that the size distribution of a nebulized MS2 aerosol, prepared using protocols similar to those shown here, will be primarily defined by the suspension contents

other than the MS2 virion itself. Whether this has an effect on the filtration behavior of the aerosol will be addressed.

The electrosprayed MS2 aerosol, shown in Figure 5-2a and in greater resolution in Figure 5-2b, possessed a d_g of 25 nm and σ_g of 1.3. A clear MS2 virion peak was observed at 24.7 nm. This was in reasonable agreement with that seen by Hogan et al. (2006) who observed a mean diameter of 24.13 nm. In that study a differential mobility analyzer (DMA) was coupled with an ultrafine condensation particle counter (CPC) which offered greater resolution than that obtained here with a lower resolution column in the DMA. This explains why our observed σ_g of 1.3 is higher than the 1.07 observed by Hogan. Electrospray was able to produce a relatively monodisperse virion aerosol with little interference from dried solutes. This is primarily due to the ability of electrospray to produce an initial droplet at or near the size of the MS2 virion. For those aerosolized droplets without a virion, they evaporate completely or form much smaller droplet nuclei.

The stability of repeat upstream aerosol particle counts at four time intervals over the 15 minute test period are presented for each generation method (Figure 5-3). The phage titer varied between prepared batches of MS2 suspension, which influenced the concentration of aerosolized virion. Therefore, the upstream particle counts were normalized for the mean of each test run to better illustrate the variability and trend over a given test sequence. Neither aerosol generation method maintained a constant particle count. Relative particle count variability of the nebulized aerosol averaged about 2.8 times that of electrosprayed aerosol. A simple linear regression was performed of the data and is presented with 95% confidence intervals for the mean and predicted particle counts. Mean particle counts from the nebulized aerosol appeared stable (though more variable) whereas the electrosprayed particle count

possessed a slight but statistically significant negative slope ($p = 0.01$) of ~ 0.16 % per minute of operation.

No statistically significant differences in filter swatch penetration were observed for the tested aerosol generation methods (see Figure 5-4). Penetration through the surgical mask was 8.3% for the electrospray aerosol and 8.0% for the Collison aerosol. Penetration through the N95 FFR sample was 0.72 and 0.70% respectively (electrospray, Collison). Electrosprayed and nebulized MS2 were likewise similar for the N100 filter sample: 0.61% for both generation methods.

Dextrose penetration was similar to MS2 for the N100 filter sample but much greater for both the surgical mask sample (12.2% compared to 8.3%) and the N95 filter sample (1.2% versus 0.73%)—a difference of 1.5 and 1.6 times greater, respectively. This was attributed to the differing dielectric properties of the MS2 virion and dextrose. As a check, we estimated the MS2 virion and dextrose particle penetration through the N95 swatch with the N95 filter characteristics described by Balazy et al. (2006b)—the same filter model used in this study—using single fiber theory for diffusion and the polarization force (Lee and Mikund 2001; Lathrace and Fissan 1986a,b). We assumed the MS2 virion's dielectric constant was 55 while that of dextrose was 3 (the dielectric constant of sucrose). We calculated an expected penetration of 0.99% for dextrose and 0.70% for the MS2 virion—a ratio of 1.4. This was in reasonable agreement with the observed values. The reason dextrose penetration did not exceed that of MS2 for the N100 filter sample is not clear. From inspection, it was apparent that the N100 sample possessed electrical charged, although the charge density was not known. It is possible the primary filtration mechanism in this filter

was diffusion since for higher filtration efficiency media, dielectrophoresis and diffusion are not additive. This would have made the effect of particle chemical composition minimal.

Despite the presence of contaminant particles and greater upstream count variability, nebulized MS2 aerosol filtration did not differ from that of the electrosprayed aerosol. This suggests that nebulized contaminant particles were filtered similarly to the MS2 virion and did not materially mask or change the aerosol's filtration behavior. Therefore, nebulization of MS2 suspension appears to be a robust method for bioaerosol filter testing over short time durations under similar conditions to those used in this study.

Both methods produced viable virus particles (Table 5-1). The higher titer and low flow of the electrospray led to a culturable aerosol concentration of ~3-fold greater (when using air) and ~20-fold greater (when using CO_2) than was obtainable with the Collison nebulizer. The PFU/cc for each aerosolization method was 95.2 for the nebulizer, 315 for the electrospray using dry filtered air, and 2118 for the electrospray using CO_2 for sheath air. Because suspension titer influences the electrosprayed aerosolized particle count, another way to compare these values is using an index of relative effectiveness by dividing the airborne culturable count with the suspension titer. This provides the PFU/cc in air per PFU/ml in the suspension. We did this then normalized the results to the nebulized aerosol. Per PFU/ml in the aerosol suspension, electrospray with CO_2 sheath air was four times as effective than the nebulizer, whereas the electrospray with regular sheath air was 60% as effective as the nebulizer. However, the applicability of this index is limited. Increasing the nebulizer suspension phage titer further could present problems. For example, the nebulizer suspension could be ultracentrifuged as is done in preparing the electrospray suspension. But, if the nebulizer suspension phage titer is too high (anecdotally >~ 10^{10} PFU/ml) aggregates

predominate in the suspension and in aerosol droplet nuclei. Therefore there is a practical upper limit to the concentration of nebulized MS2.

5.4 CONCLUSIONS FOR COROLLARY TO SPECIFIC AIM 3

The MS2 virus solution used for the nebulizer required fewer steps, could be completed is a shorter time, but resulted in a lower titer solution than the protocol used to prepare the electrospray suspension. The electrospray suspension required several additional purification and concentration steps which resulted in solution at least 100 times more concentrated than that used by the nebulizer.

The nebulizer produced a polydisperse aerosol while that produced by electrospray was relatively monodisperse, with a clear particle count peak at the electrical mobility size of the single MS2 virion. The nebulized aerosol count was dominated by the presence of contaminants in the aerosol. Neither aerosolization method was able to maintain a constant concentration over 15 minute test periods. The nebulized aerosol particle count was 2.8 times as variable around the count mean than the electrosprayed aerosol. Despite this, the mean particle count from the nebulized aerosol was constant throughout repeated tests while the electrosprayed aerosol possessed a slight negative slope.

No differences in filter penetration were observed for MS2 aerosolized by nebulization and electrospray. Interestingly, the presence of contaminants in the nebulized MS2 aerosol did not appear to influence filtration behavior. Electrosprayed dextrose particles were more penetrating than MS2 particles in two of the three filter samples, which was attributed to its dielectric properties. Both aerosolization methods produced culturable MS2 virion with the

electrospray producing approximately 20 times more than nebulization when using CO_2 as the electrospray sheath air.

The electrospray appears to produce a unique aerosol in terms of virion purity and variability of particle count, which may have application in processes requiring a very clean biological aerosol in the ultrafine size range. The findings of this study are expected to assist researchers in selecting appropriate generation methods when using viable virus- and bacteria-based challenge aerosols.

DISCUSSION AND OVERALL CONCLUSIONS

This dissertation expands and contributes to the scientific literature in several important ways. First, this dissertation at minimum refines and arguably redefines the “most conservative” scenario for use in respirator filter testing and certification. This dissertation has shown that the assumed most-penetrating particle size utilized for respirator filter certification is too large when testing electret filters with neutralized aerosol at physiologically relevant airflows. It was also shown that the true most-penetrating particle size under these conditions is either not measured or poorly measured in the current certification protocol.

Second, this dissertation clearly defined the lower boundary of particle size detection of the existing NIOSH certification protocol. Despite its *prima facie* importance to health, this lower boundary has not previously been defined and illustrated in the literature.

Third, this dissertation provided insight into several notable characteristics of ultrafine biological particles which may cause their filtration behavior to differ from inert surrogates. Ultrafine biological particles were observed to have less penetration through electret filters than low dielectric constant surrogates. This also helps to refine a “most conservative” filter testing scenario, because low dielectric constant, inert surrogates can be expected to have equal or greater penetration through electret filters as biological particles.

Fourth, a method was developed and tested that may be used to assess a new class of “antimicrobial” respirator filters. As more of these new respirators are developed and marketed, the ability to test claims of antimicrobial filtration will be a useful tool.

Last, this dissertation performed a comparison of nebulization and electrospray for aerosolizing virions in filter testing. It was demonstrated that, despite the shortcomings of the

nebulized aerosol, when repeated measures are used over short duration tests, filter test results can be reliable and comparable to a nearly monodisperse virion aerosol. This last study will provide a useful comparator and guide for when to use nebulization or electrospray for aerosolizing virions and possibly other bioaerosols.

FUTURE DIRECTIONS

This work identifies a knowledge gap in terms of respiratory protection against ultrafine particles. In particular, ultrafine particles with unique dielectric properties. Biological aerosols will possess dielectric properties close that of water droplets, with relatively high dielectric constants (~> 60). Engineered nanomaterials may possess much higher dielectric constants. This implies such materials would be filtered very efficiently by electret filters. A question arises as to how these more exotic materials would effect filter efficiency after loading. Whether such materials would degrade electret filter efficiency is an interesting question that deserves to be pursued.

This work focused on respirator filter penetration, but total inward leakage (TIL) of ultrafine particles deserves significant attention. Many larger particles are both filtered out and do not pass through leaks because of interception and impaction mechanisms. Smaller particles are more likely to follow airflow streamlines and penetration through respirator face seal leaks. This has been studied somewhat for larger particles but deserves attention for submicrometer particles, particularly particles in the ultrafine size range. I would hypothesize that total inward leakage of particles increases with decreasing particle size.

This study relied on the concept of the “single airborne virion” using bacteriophage MS2 as a model. However, this approach is debatable as many public health professionals believe that infectious disease is transmitted by larger droplet aerosols. It would be interesting to pursue study that provided insight as to the likelihood of expired droplets evaporating to droplet nuclei and their fate in a hospital environment, for example. Perhaps

even detecting size-fractioned viable influenza particles from animal models would go far in answering this question.

All filter tests in this dissertation utilized constant flow conditions. However, cyclic flow conditions may provide a better estimate of real-world respirator filter penetration. This is a question that is being pursued in this laboratory by another researcher, and it will be interesting to see if there are practical differences between testing respirators with constant flow compared to cyclic flow.

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TABLES

Table 2-1. Non-Powered Particulate Air-Purifying Respirator Classification (summary)

Respirator	Minimum Filtration Efficiency %	Challenge Aerosol	Max Filter Loading	Usage Limitation	Certification Preconditioning[C]	Certification Flowrate
N95	95	NaCl	200 mg	Non-oil aerosols only	Y	
R95	95	DOP	200 mg	8-hrs/one workshift[A]	N	
P95	95	DOP	Lowest efficiency	Per user instructions[B]	N	
N99	99	NaCl	200 mg	Non-oil aerosols only	Y	
R99	99	DOP	200 mg	8-hrs/one workshift[A]	N	85 lpm
P99	99	DOP	Lowest efficiency	Per user instructions[B]	N	
N100	99.97	NaCl	200 mg	Non-oil aerosols only	Y	
R100	99.97	DOP	200 mg	8-hrs/one workshift[A]	N	
P100	99.97	DOP	Lowest efficiency	Per user instructions[B]	N	

A. In oil aerosol environment.

B. P-series respirator filter service life recommendations are manufacturer specific.

C. N-series filters require preconditioning at 85% relative humidity and 38^{o}C for 25 hours.

Table 2-2. NIOSH Challenge Aerosol Characteristics for Particulate Respirator Filtration

Challenge Aerosol	Density	Count Median Diameter (CMD)[A]	Geometric Std Deviation (GSD)	Mass Median Diameter (MMD)[C]	Mass Median Aerodynamic Diameter (MMAD)
Sodium Chloride, NaCl	2.13	75 ± 20 nm	≤ 1.86	238 nm	347 nm
% count distribution:[b]			% mass distribution:[b]		
± 1 SD (68%)		40 – 140 nm	± 1 SD (68%)	128 – 443 nm	
± 2 SD (95%)		22 – 252 nm	± 2 SD (95%)	70 – 732 nm	
Dioctylphthalate, DOP	0.986	165 ± 20 nm	≤ 1.60	359 nm	356 nm
% count distribution:[b]			% mass distribution:[b]		
± 1 SD (68%)		116 – 295 nm	± 1 SD (68%)	224 – 560 nm	
± 2 SD (95%)		73 – 464 nm	± 2 SD (95%)	142 – 821 nm	

A. Per 42 CFR 84.181(g), the challenge aerosol CMD must be within +/- 20 nm.
B. Calculated using Hatch-Choate equations [6].
C. Count, mass distributions differ slightly from those predicted by the logarithmic function due to assumed 1 µm upper particle size.

Table 2-3. Percent Contribution by Size for Two Challenge Aerosols

Particle Size Range	NaCl Test Aerosol (%)[A]			DOP Test Aerosol (%)[A]		
nm	Count	Mass	Scatter	Count	Mass	Scatter
0 – 100	68	8	0.6[B*]	10	0.3	<0.01[B]
100 – 200	26	31	8	47	11	2
200 – 300	4	26	20	28	25	12
300 – 400	1	15	25	10	24	24
400 – 500	0.2	9	23	3	17	28
500 – 600	0.07	5	13	1	10	19
600 – 700	0.02	3	7	0.4	6	10
700 – 800	<0.01	2	3	0.1	3	5
800 - 900	<0.01	1	0.7	0.05	2	1
900 - 1000	<0.01	0.6	0.3	0.02	1	0.4

A. Columns may not add to 100% due to rounding error.

B. Scatter values for 0 – 100 nm are theoretical; scatter from ultrafine particles is poorly detected by photometers

Table 2-4. Summary of Studies: Electret Filter Penetration and Most-Penetrating Particle Size

Study	Filter/Respirator Properties[A]					Challenge Aerosol (Charge Condition)	V_f[B] (cm/s)	MPPS[C] (nm)	Comments
	Material	α	d_f (µm)	L (mm)	q or σ				
Baumgartner & Loffler, 1986	Split fiber Spun fiber		ρ_{sf} = 250 g/m^2 ρ_{sf} = 30 g/m^2		Not provided	NaCl, 20 – 250 nm (Boltzmann)	10	< 100	Filter properties not provided
Lathrace & Fissan, 1986	Split fiber Spun fiber	0.042 0.035	30 5.2	5 1	2e-4 – 5e-4 C/m^2 8e-3 – 0.2 nC/m	NaCl, 20 – 189 nm DES, 140 nm – 1 µm (Boltzmann)	2 – 30	< 100	MPPS > 100 nm in 2 of 6 test conditions
Kanaoka *et al*., 1987	PP	0.031 0.075	~24*	4 5	34.2 nC/m	NaCl, 20 – 400 nm, (Uncharged, Boltzmann)	5 – 200	<50	*Rectangular fibers
Stevens & Moyer, 1989	Commercially available dust/mist, paint/lacquer/enamel/mist, dust/fume/mist, and high efficiency (HE) respirator filters; filter materials: wool, wool resin, electrostatic felt, felt resin, fiberglass					NaCl,30 – 240 nm; DOP, 30 – 300 nm (Boltzmann)	Q = 16 – 85 L/min	55 – 120	MPPS of all tested HE filters 90 – 175 nm
Brosseau et al., 1989	10 commercially available dust/mist respirators filters; 9 were combined resin-impregnated wool and PP felt, 1 was PP					Latex spheres, 102 nm – 2.02 µm (Boltzmann)	55.3 – 74.8	≤ 102	Q = 2.7 L/min; filter surface area range: 36.1 – 48.8 cm^2
Fardi & Liu, 1991	1 mechanical dust/mist and 2 electret dust/mist or dust/mist/fume respirator filters					NaCl, DOP 35 nm – 4 µm (Boltzmann)	Q = 16, 32, 45 L/min	~ 100	Face velocity or filter surface area not provided

A. PP = polypropylene; α = filter packing density; d_f = fiber diameter; L = thickness; q (nC/m) or σ (C/m^2) = charge density; ρ_{sf} = surface density.
B. V_f = filter face velocity; provided where given or can be calculated from data provided in study; **Q** = volumetric flowrate.
C. MPPS = most-penetrating particle size.

Table 2-4. Summary of Studies: Electret Filter Penetration and Most-Penetrating Particle Size (continued)

Study	Filter/Respirator Properties[A]					Challenge Aerosol (Charge Condition)	V_f[B] (cm/s)	MPPS[C] (nm)	Comments
	Material	α	d_f (μm)	L (mm)	q or σ				
Martin & Moyer, 2000	6 commercially available models of FFR: 3 N95, 1 N99, 1 R95, 1 P100					NaCl, DOP ~ 25 – 400 nm (Boltzmann)	Q = 85 L/min	50 – 100	Aerosols complied with 42 CFR 84.181; filter surface area not provided
Oh et al., 2002	Spun filter	0.04	9		1.44 nC/m	NaCl (Boltzmann)	10	~ 85	Numerical simulation; good agreement with Baumgartner & Loffler
Balazy *et al*. 2006a	PP FFR PP FFR	0.069 0.091	7.84 7.19	1.77 0.35	13 – 14 nC/m* Not provided	NaCl, ~ 10 – 600 nm (Boltzmann)	4.5, 12.9 3.7, 10.6	40 – 50	2 models of N95 FFR; *q was estimated
Balazy *et al* 2006b	Same as Balazy *et al*. 2006a.					MS2 bacteriophage, ~30 – 80 nm (Boltzmann)	4.5, 12.9 3.7, 10.6	40 – 60	Same as Balazy *et al*. 2006a.
Richardson *et al*., 2006	Commercially available devices: 2 N95 FFR, 2 P100 FFR, 2 N95 cartridges, 2 P100 cartridges					NaCl, DOP 20 nm – 3.02 μm; PSL .7 – 2.9 μm (Boltzmann)	0.7 - 60 See comments	50 - 100	MPPS for P100 > 100 nm; cyclic (40 – 135 L/min) and constant flow (42.5 – 180 L/min) test conditions
Rengasamy *et al*., 2007	5 commercially available models of N95 FFR					NaCl 20 – 400 nm (Boltzmann)	Q = 85 L/min	40	Filter surface area not provided

A. PP = polypropylene; α = filter packing density; d_f = fiber diameter; L = thickness; q (nC/m) or σ (C/m^2) = charge density; ρ_{sf} = surface density.
B. V_f = filter face velocity; provided where given or can be calculated from data provided in study; **Q** = volumetric flowrate.
C. MPPS = most-penetrating particle size.

Table 3-1. Summary of aerosol penetration (P), pressure drop, and quality factor (q_f) for three respirators.

Aerosol: NaCl

Respirator	Q (L/min)	Pressure Drop (mmH_2O)	$P_{0.1\mu m}$ (%)	$q_{f\ 0.1\mu m}$ ($1/mmH_2O$)	$P_{0.3\mu m}$ (%)	$q_{f\ 0.3\mu m}$ ($1/mmH_2O$)	$P_{0.02\text{-}0.5\mu m}$ (%)	$q_{f\ 0.02\text{-}0.5\mu m}$ ($1/mmH_2O$)	$P_{0.1\text{-}0.5\mu m}$ (%)	$q_{f\ 0.1\text{-}0.5\mu m}$ ($1/mmH_2O$)
N99 model A	30	3.90 ± 0.20	0.66 ± 0.04	1.29 ± 0.06	0.35 ± 0.03	1.45 ± 0.10	0.75 ± 0.04	1.29 ± 0.06	0.43 ± 0.03	1.41 ± 0.08
	85	10.67 ± 0.58	2.92 ± 0.46	0.33 ± 0.03	0.99 ± 0.24	0.44 ± 0.05	3.20 ± 0.46	0.34 ± 0.03	1.60 ± 0.30	0.40 ± 0.04
	150	24.33 ± 2.08	5.14 ± 0.58	0.12 ± 0.01	2.07 ± 0.26	0.16 ± 0.01	5.93 ± 0.61	0.12 ± 0.01	3.13 ± 0.38	0.15 ± 0.01
N99 model B	30	4.53 ± 0.15	0.74 ± 0.10	1.08 ± 0.06	0.44 ± 0.22	1.21 ± 0.09	0.56 ± 0.11	1.20 ± 0.05	0.56 ± 0.18	1.17 ± 0.07
	85	13.00 ± 1.00	2.78 ± 0.34	0.28 ± 0.02	1.27 ± 0.50	0.34 ± 0.05	2.36 ± 0.20	0.31 ± 0.02	1.65 ± 0.14	0.32 ± 0.02
	150	24.67 ± 1.15	4.87 ± 0.94	0.12 ± 0.01	3.07 ± 1.96	0.15 ± 0.02	4.23 ± 1.27	0.13 ± 0.01	3.60 ± 1.53	0.14 ± 0.01
N95	30	2.70 ± 0.10	0.83 ± 0.20	1.78 ± 0.10	0.48 ± 0.14	1.99 ± 0.15	0.87 ± 0.21	1.80 ± 0.10	0.66 ± 0.21	1.89 ± 0.14
	85	7.57 ± 0.75	2.60 ± 0.51	0.49 ± 0.02	1.34 ± 0.44	0.58 ± 0.02	2.85 ± 0.44	0.49 ± 0.03	1.74 ± 0.41	0.54 ± 0.03
	150	15.83 ± 2.02	4.65 ± 0.48	0.20 ± 0.03	2.84 ± 0.38	0.23 ± 0.04	5.16 ± 0.35	0.19 ± 0.03	3.42 ± 0.46	0.26 ± 0.03

Aerosol: MS2

Respirator	Q (L/min)	$P_{0.02\text{-}0.09\mu m}$ (%)
N99 model A	30	1.03 ± 0.55
	85	3.43 ± 0.86
	150	5.45 ± 0.35
N99 model B	30	0.96 ± 0.12
	85	3.28 ± 0.20
	150	5.70 ± 0.61
N95	30*	1.69 ± 0.38
	85*	3.45 ± 0.48
	150	5.64 ± 1.94

Aerosol: *Bacillus subtilis* phage

Respirator	Q (L/min)	$P_{0.1\mu m}$ (%)
N95	30	0.58 ± 0.22
	85	1.90 ± 0.19
	150	3.81 ± 0.60

Aerosol: T4 phage

Respirator	Q (L/min)	$P_{0.1\mu m}$ (%)
N95	30	0.23 ± 0.01
	85	0.95 ± 0.11
	150	2.18 ± 0.37

*MS2 data for the N95 respirator are taken from Balazy *et al*., 2006b for 30 and 84 L/min

Table 5-1. Summary: Culturable Bacteriophage Comparison

Method	Suspension Titer (PFU/ml)	Aerosol Conc (PFU/cc_{air})	Relative Effectiveness[1]
Nebulizer	2.2×10^9	95.2	1
Electrospray – air	1.2×10^{10}	315	0.6
Electrospray – CO_2	1.2×10^{10}	2118	4

[1] PFU/cc in air per PFU/ml in the suspension, normalized to the nebulizer

FIGURES

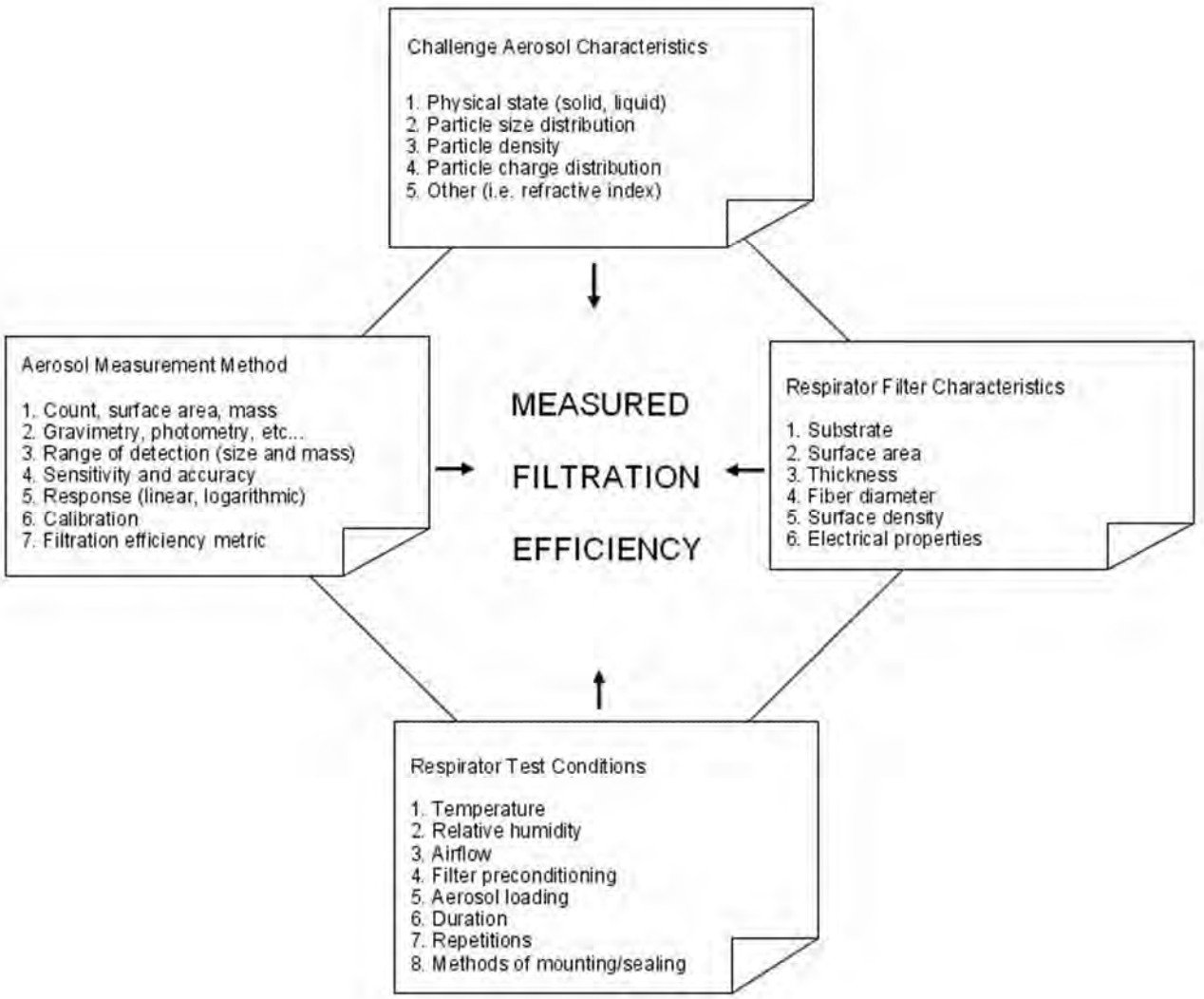

Figure 2-1. Factors influencing respirator filter test results

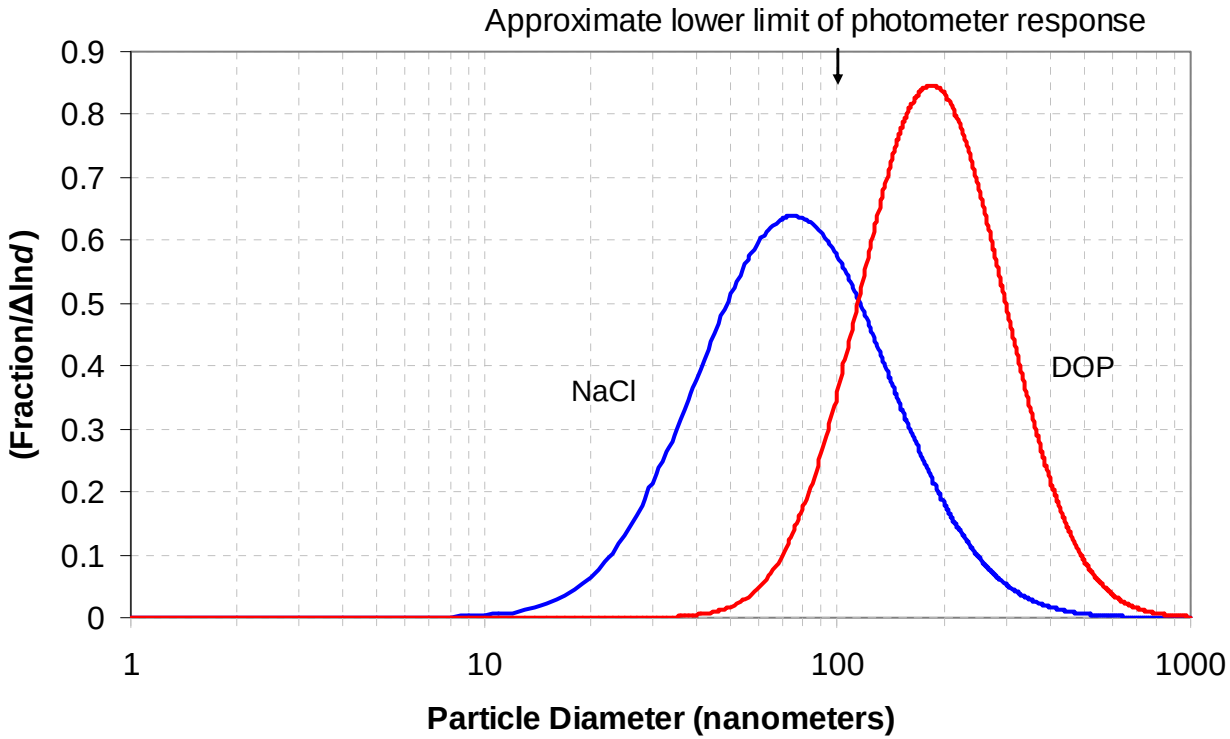

Figure 2-2. Challenge aerosol particle size distributions (by count) and photometer limit of detection

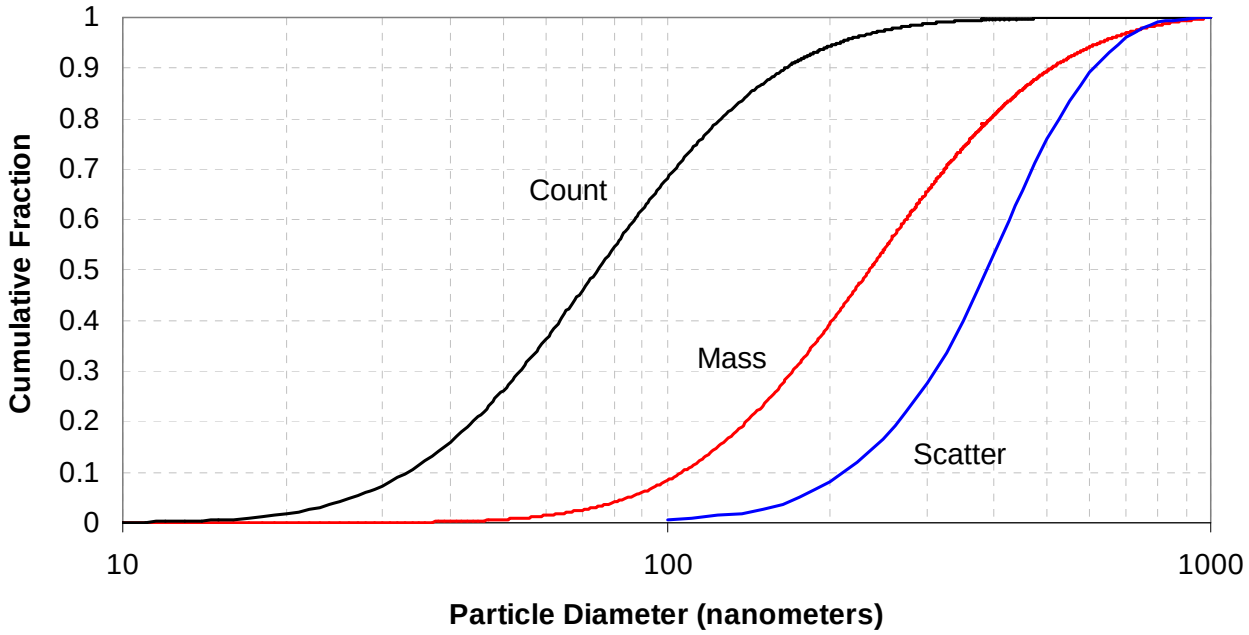

Figure 2-3a. NaCl challenge aerosol cumulative fractions: count, mass, light Scatter

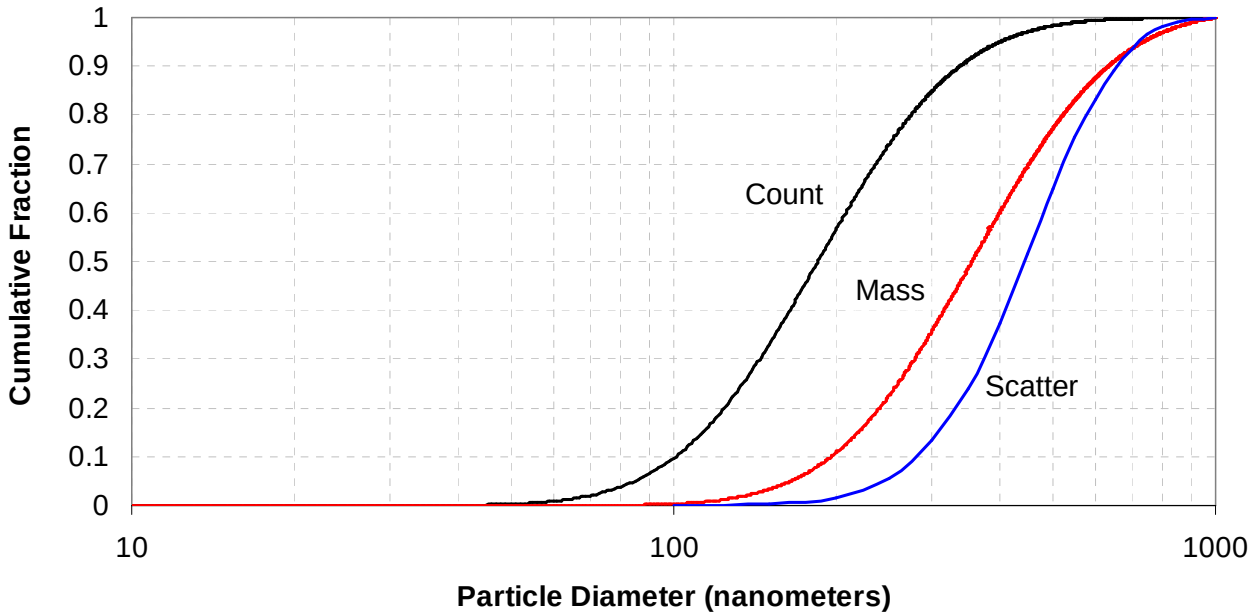

Figure 2-3b. DOP challenge aerosol cumulative fractions: count, mass, light scatter

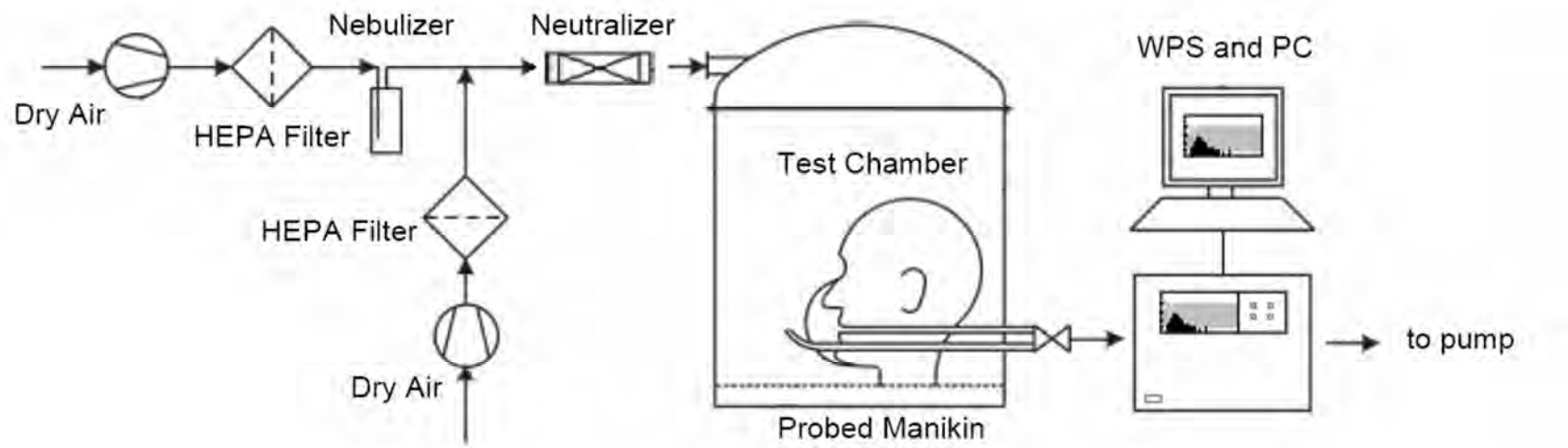

Figure 3-1. Filter penetration test system. Diagram adapted from Balazy *et al.* (2006a,b).

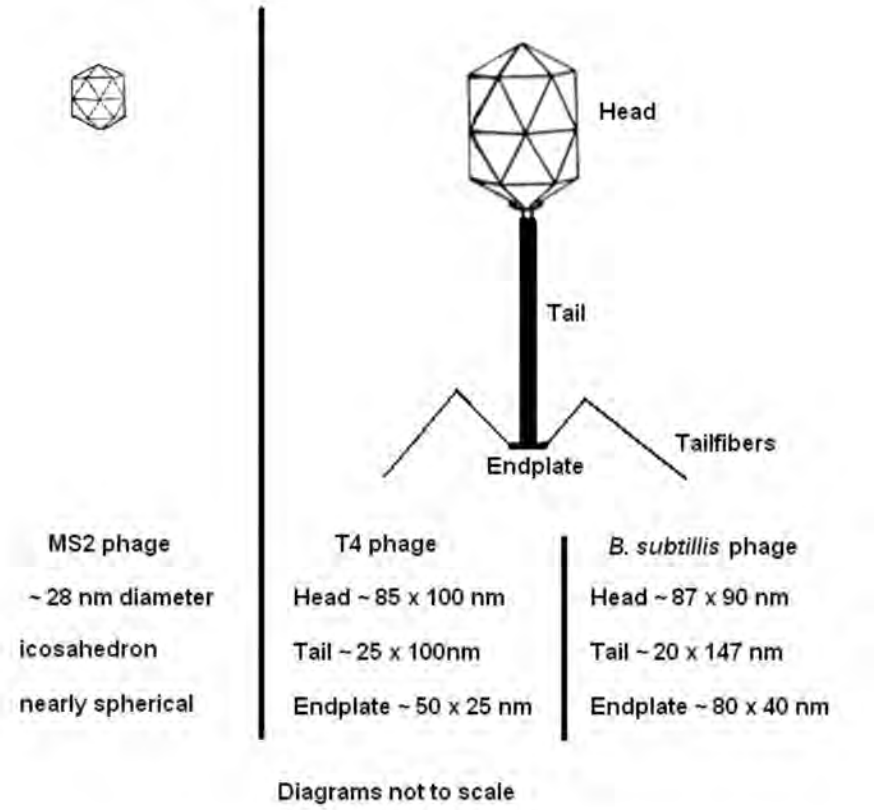

Figure 3-2. Shape and dimensions of the bacteriophages used in this study.

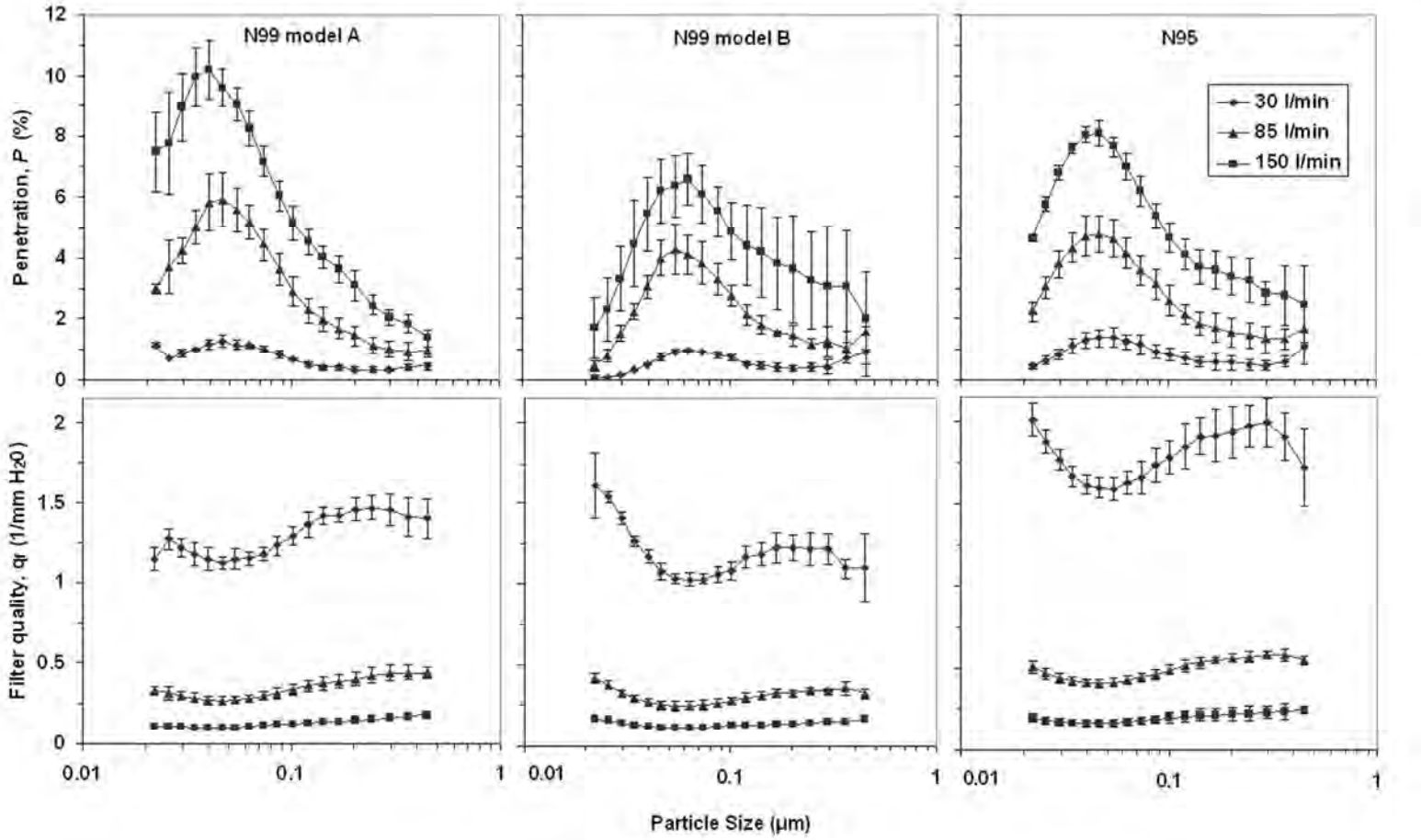

Figure 3-3. Aerosol penetration and filter quality factor of three respirators as a function of the particle size and inhalation flow rate for NaCl challenge aerosol.

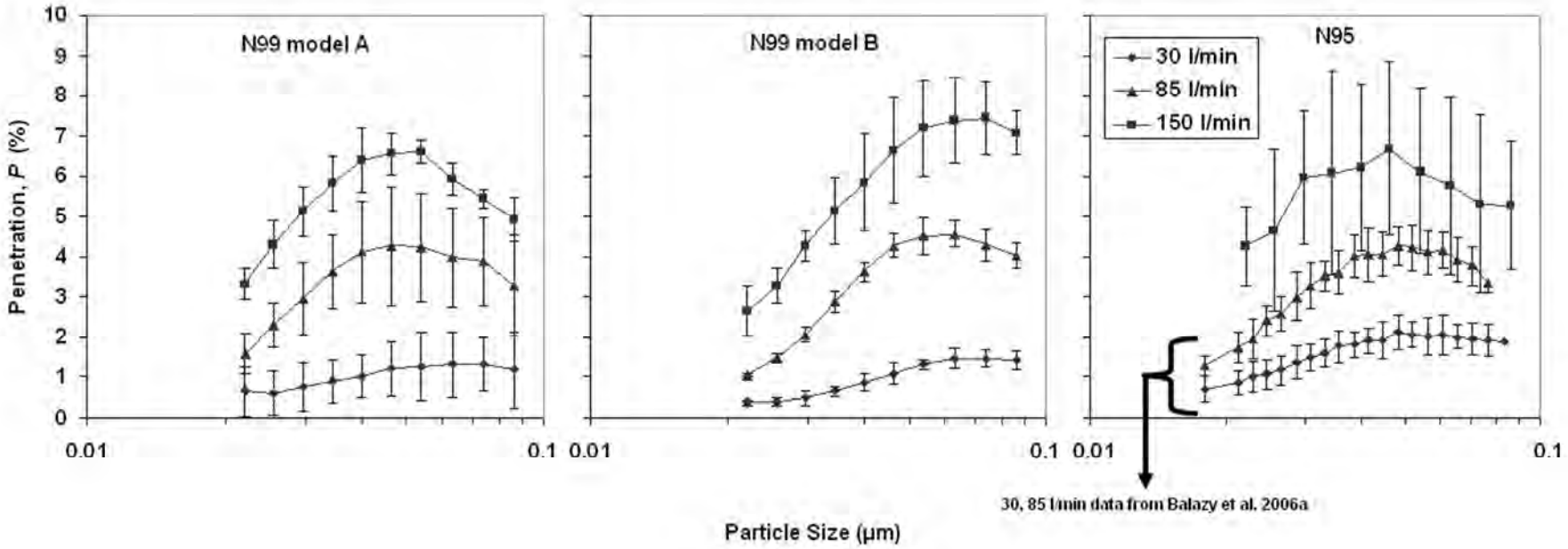

Figure 3-4. Aerosol penetration through three respirators as a function of the particle size and inhalation flow rate for MS2 bacteriophage challenge aerosol.

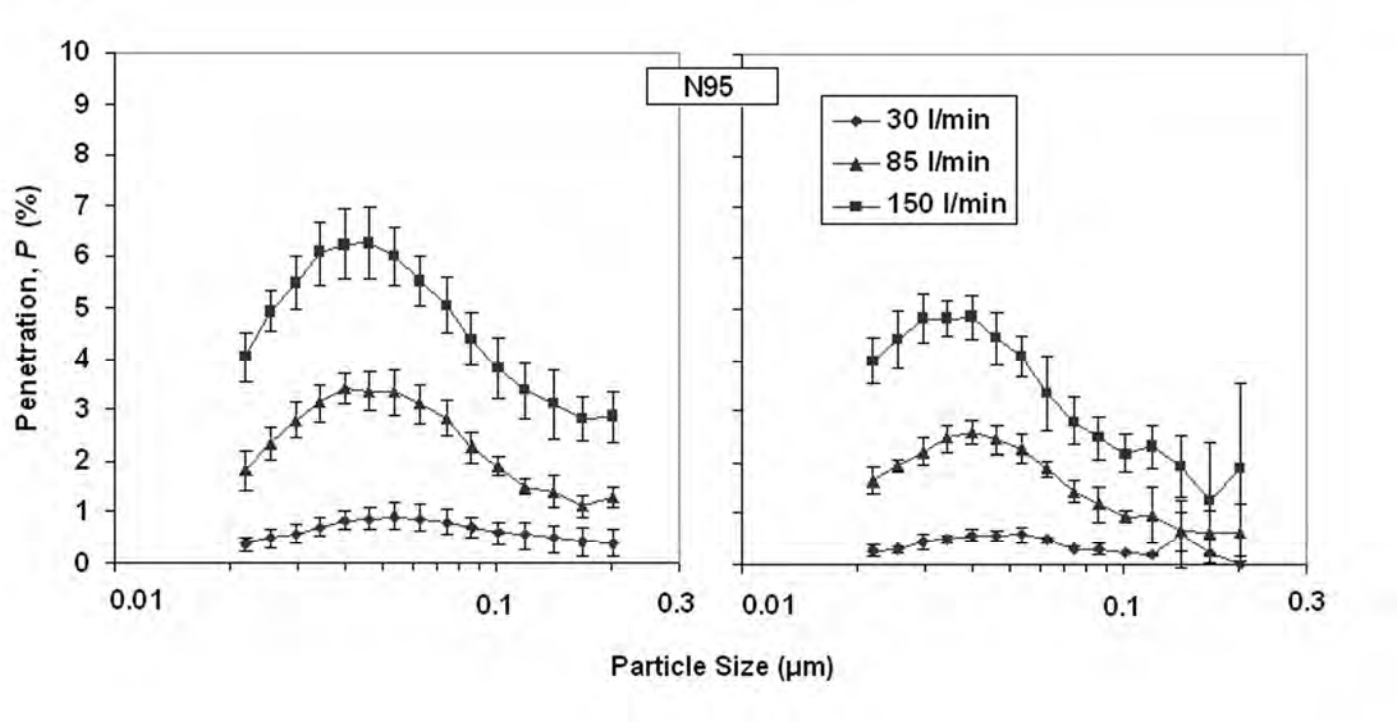

Figure 3-5. Aerosol penetration through N95 respirator as a function of the particle size and inhalation flow rate for two challenge viruses: *B. subtilis* bacteriophage (left) and T4 bacteriophage (right).

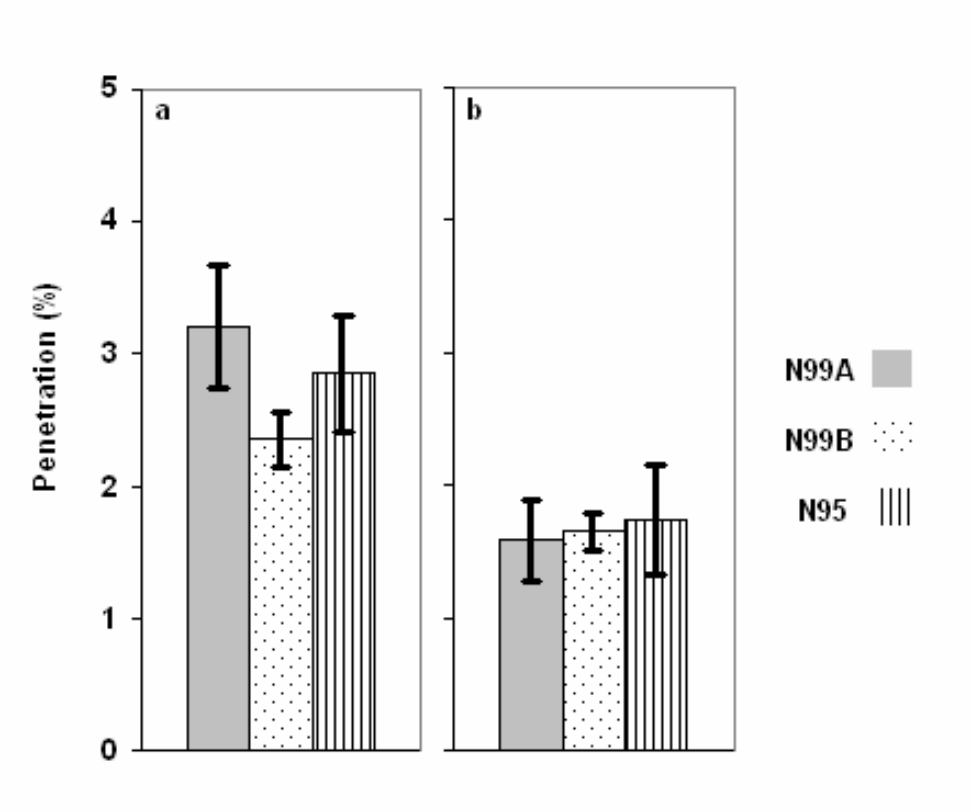

Figure 3-6. Between respirator comparison: mean penetration of NaCl [integrated for the size range of 0.02–0.5 µm (a), and 0.1–0.5 µm (b)] at 85 L/min.

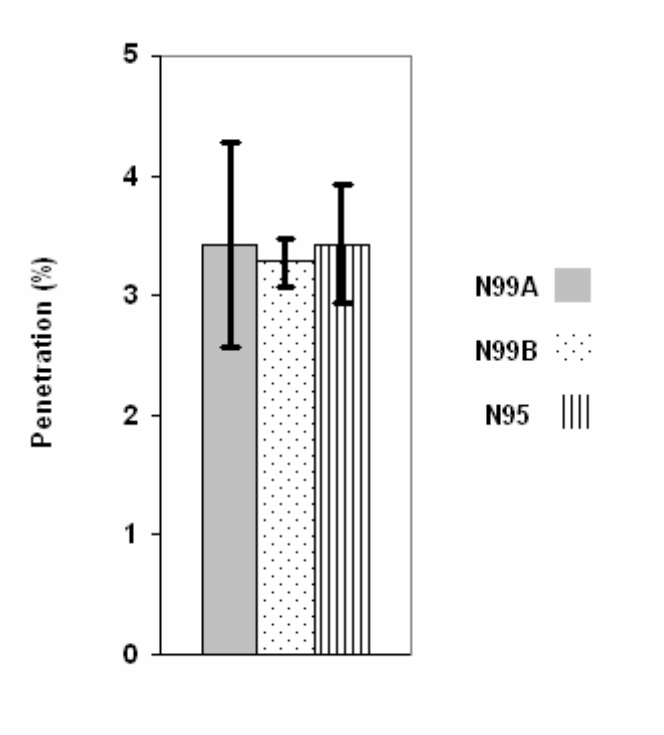

Figure 3-7. Between respirator comparison: mean penetration of MS2 (integrated for the size range of 0.02 – 0.09 µm) at 85 L/min.

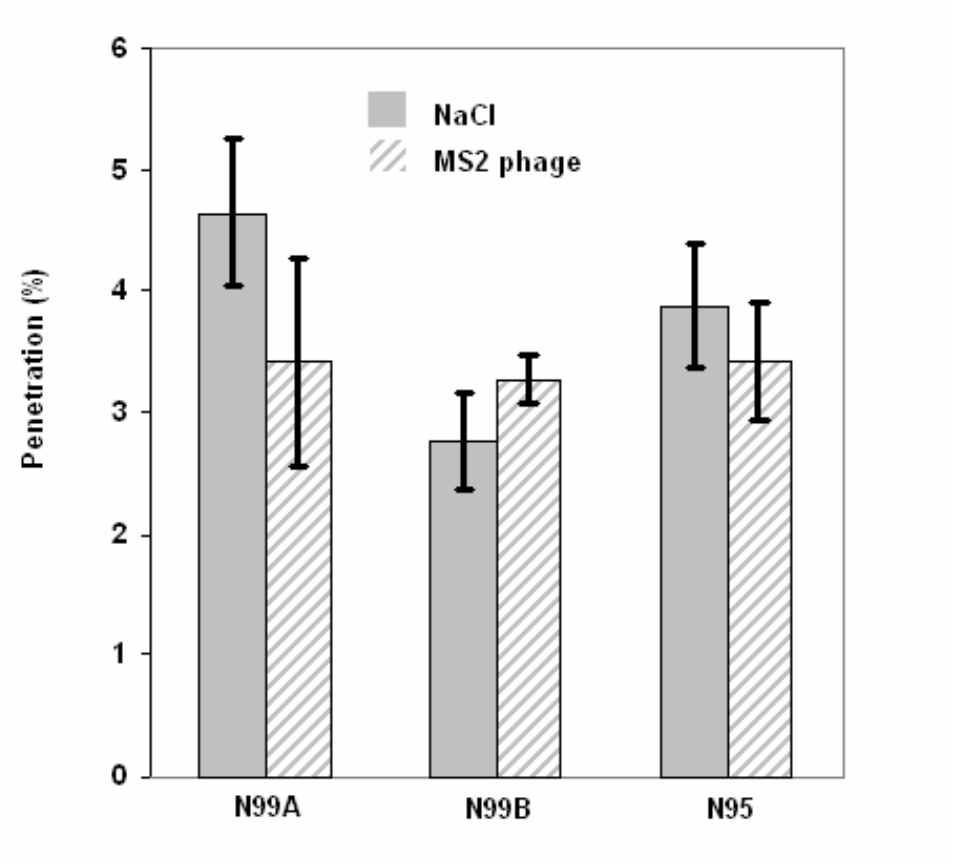

Figure 3-8. Within respirator comparison: mean penetration of NaCl and MS2 (integrated for the size range of 0.02 – 0.09 µm) at 85 L/min.

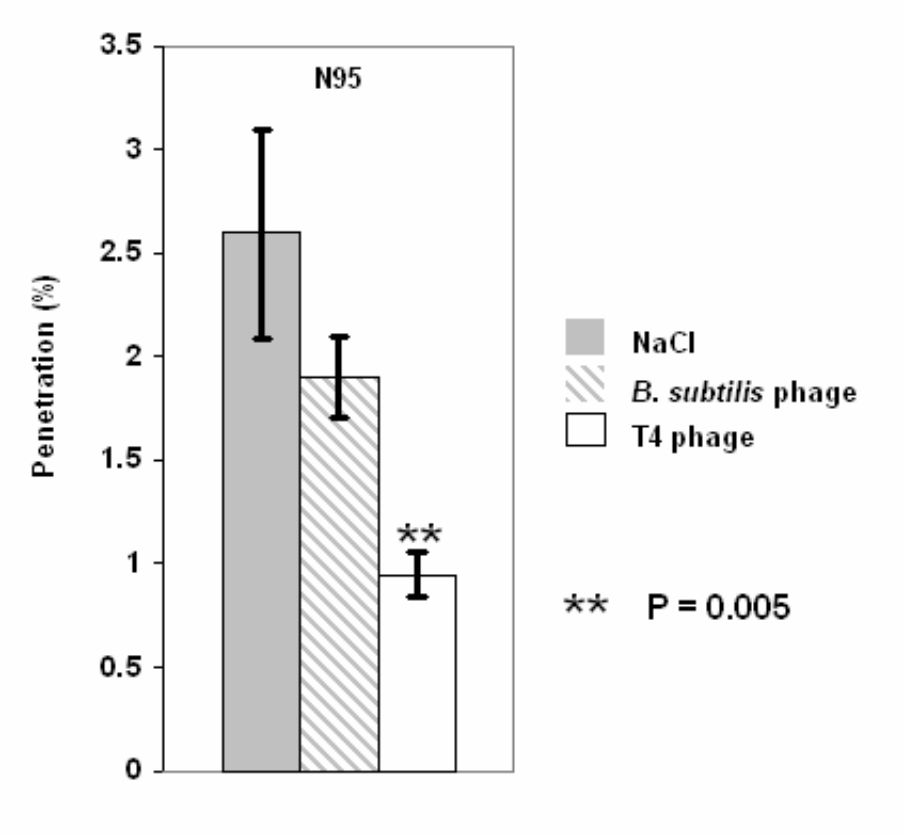

Figure 3-9. Within respirator comparison for N95 at 85 L/min: mean penetration of NaCl compared to *B. subtilis* phage and T4 phage at 0.1 µm.

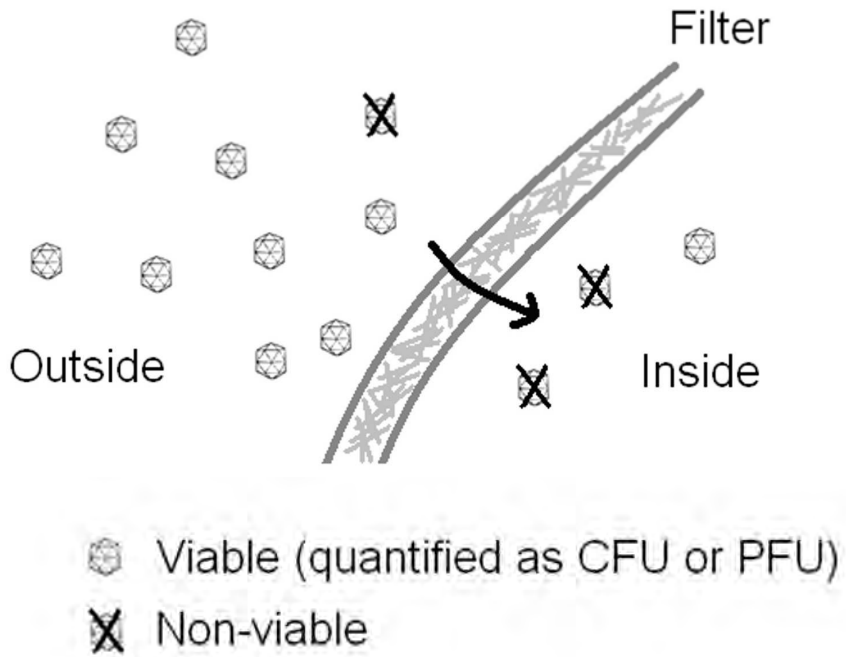

Figure 4-1. Illustration of physical and viable filtration.

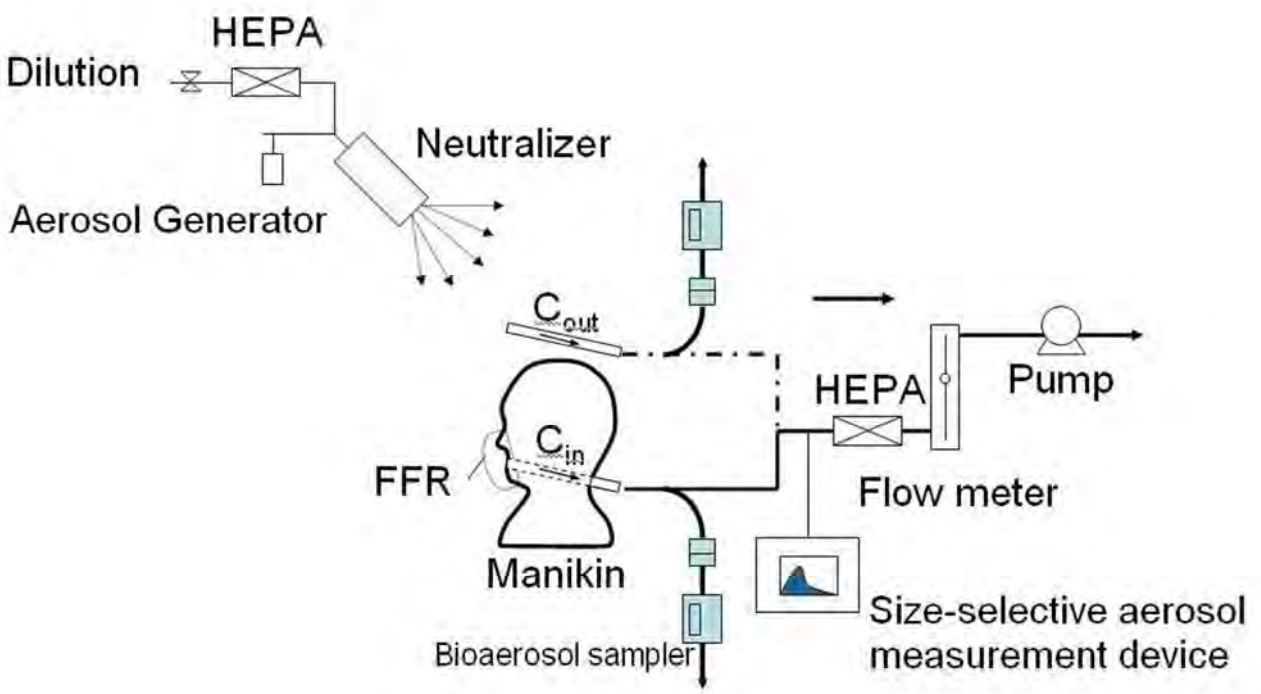

Figure 4-2. Setup for testing physical and viable filtration efficiencies.

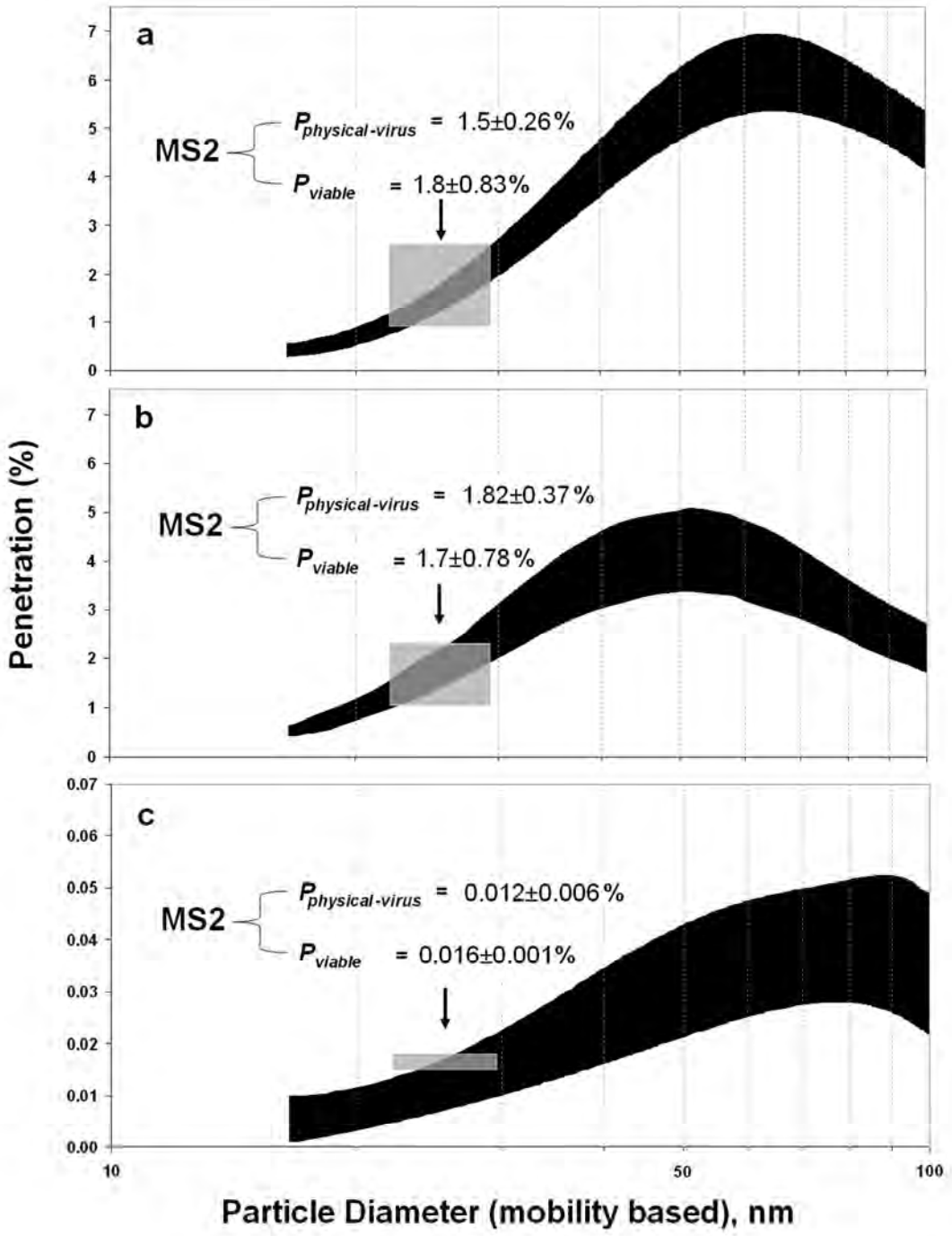

Figure 4-3. Size-fractioned $P_{physical}$ (black) superimposed by P_{viable} (grey). $P_{physical\text{-}virus}$ was determined specifically for the virus-designated mobility-based diameter of 22 – 29 nm. The plots are bounded by the mean penetration ± 1 SD. Figure 3a: conventional N95 respirator #1; Figure 3b: conventional N95 respirator #2; Figure 3c: iodinated polymer respirator P95.

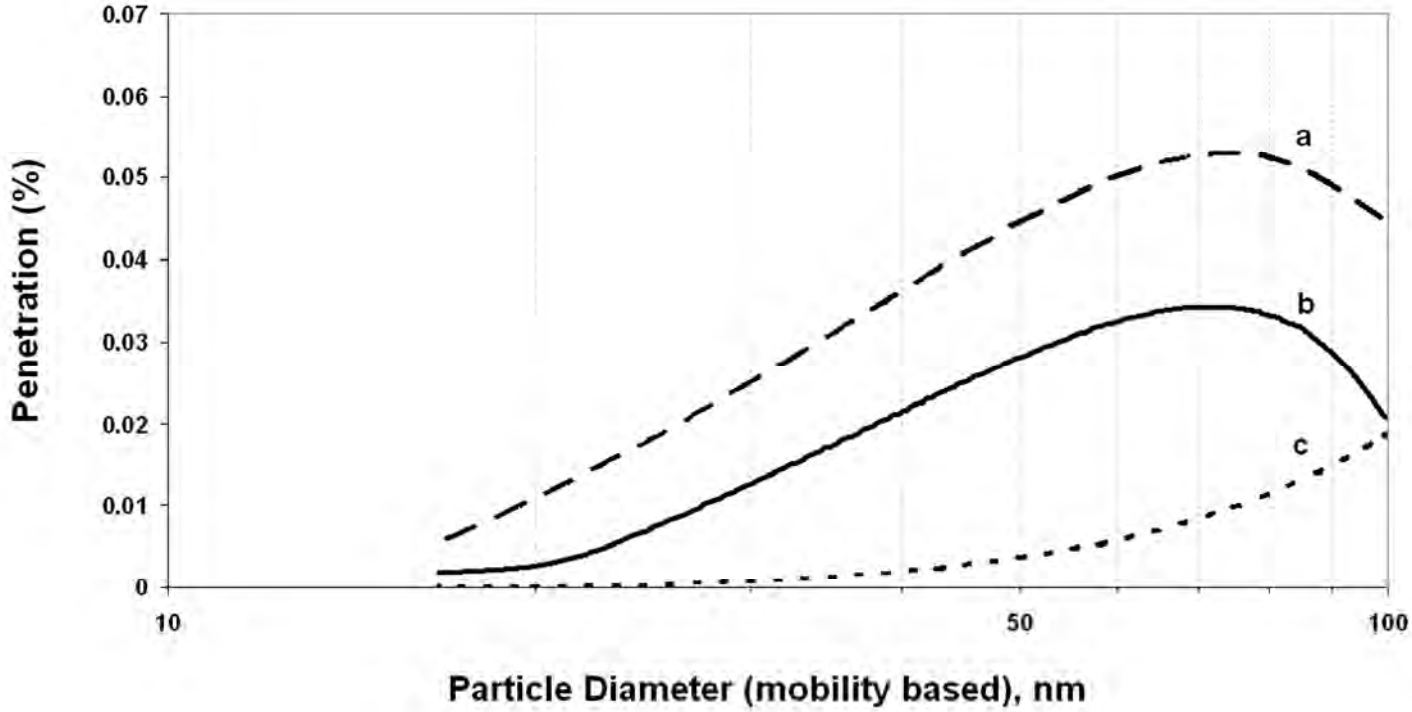

Figure 4-4. Size-fractioned physical penetration of NaCl for the (a) P95 filter swatch with no iodinated resin; (b) commercially-available P95 respirator filter (10 g/m^2); and (c) P95 filter swatch highly treated with iodinated resin (35 g/m^2). Lines are best-fit polynomial regressions of the data with R^2 values of 0.70, 0.97, and 0.98, respectively.

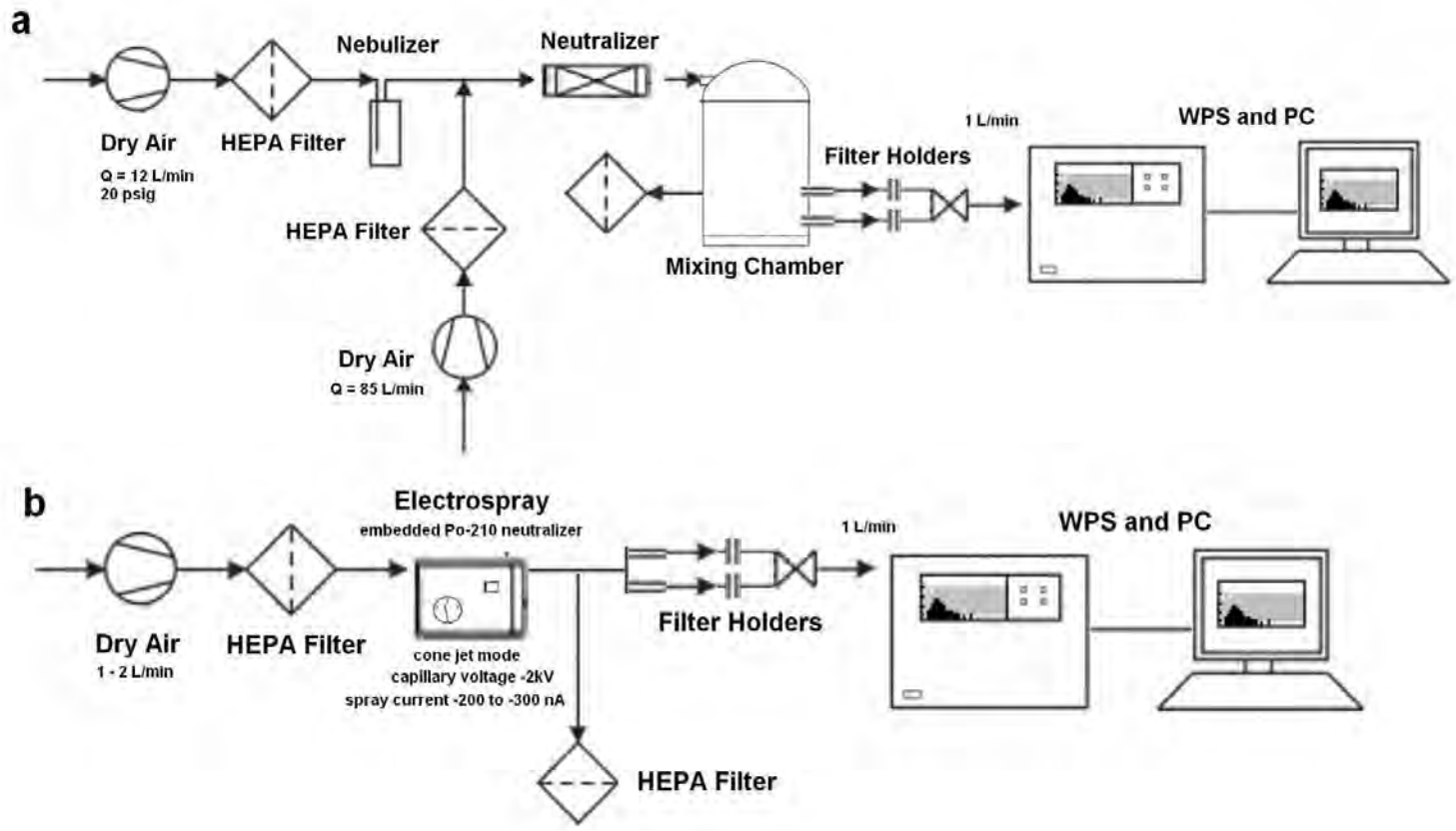

Figure 5-1. Experimental Setup: Filter Testing

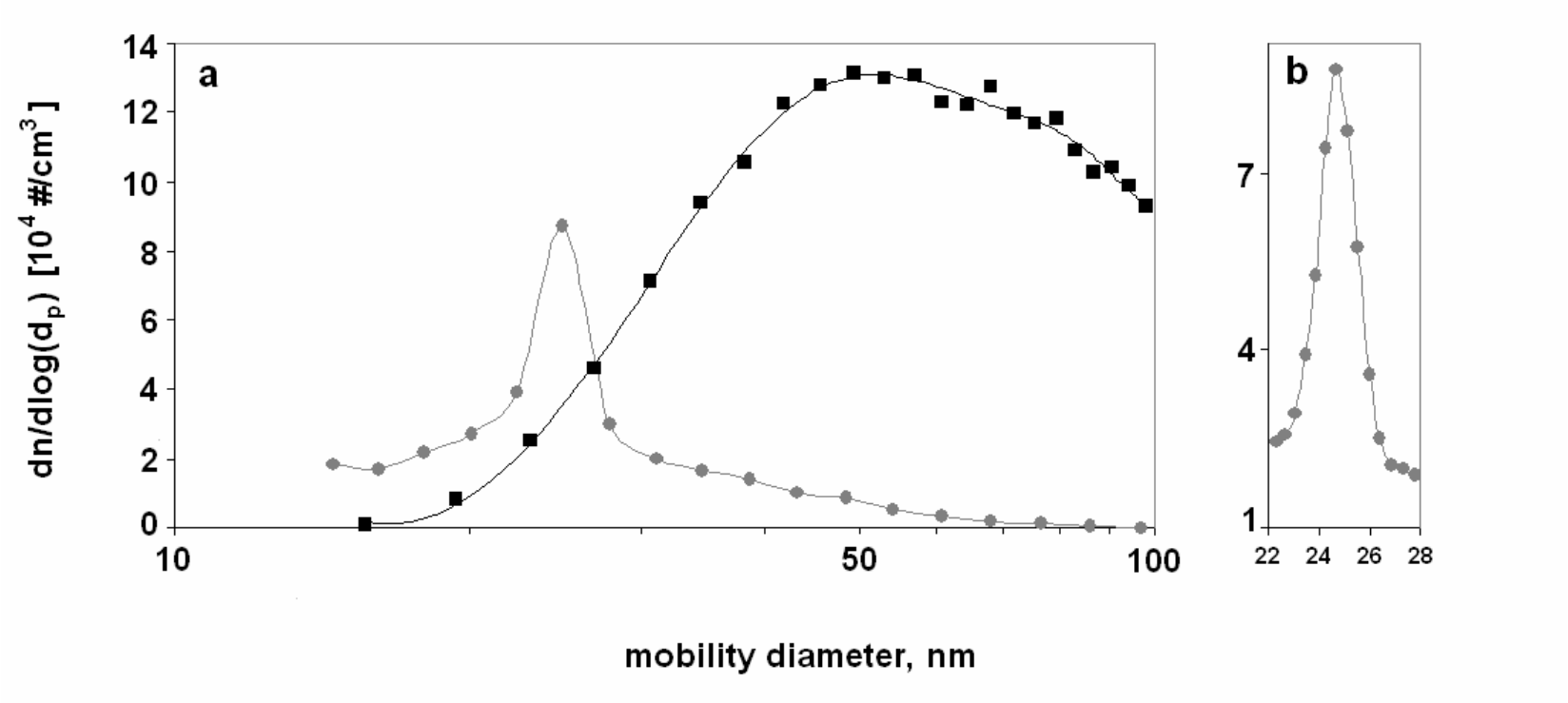

Figure 5-2. MS2 aerosol particle size distribution: (a) both aerosols (nebulizer in black); (b) electrosprayed size distribution magnified.

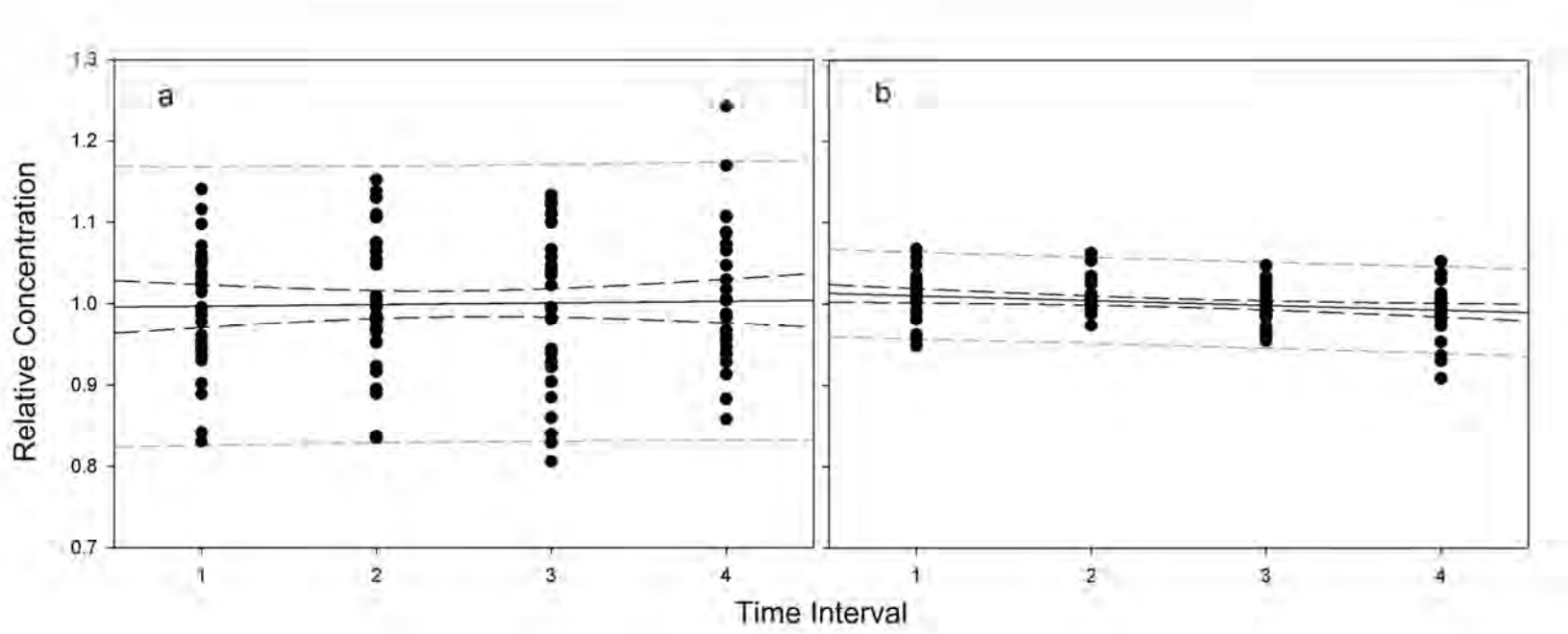

Figure 5-3. Particle count variability and trend over 15 minute filter tests, normalized to the mean of each test run. (a) Collison nebulizer; (b) electrospray.

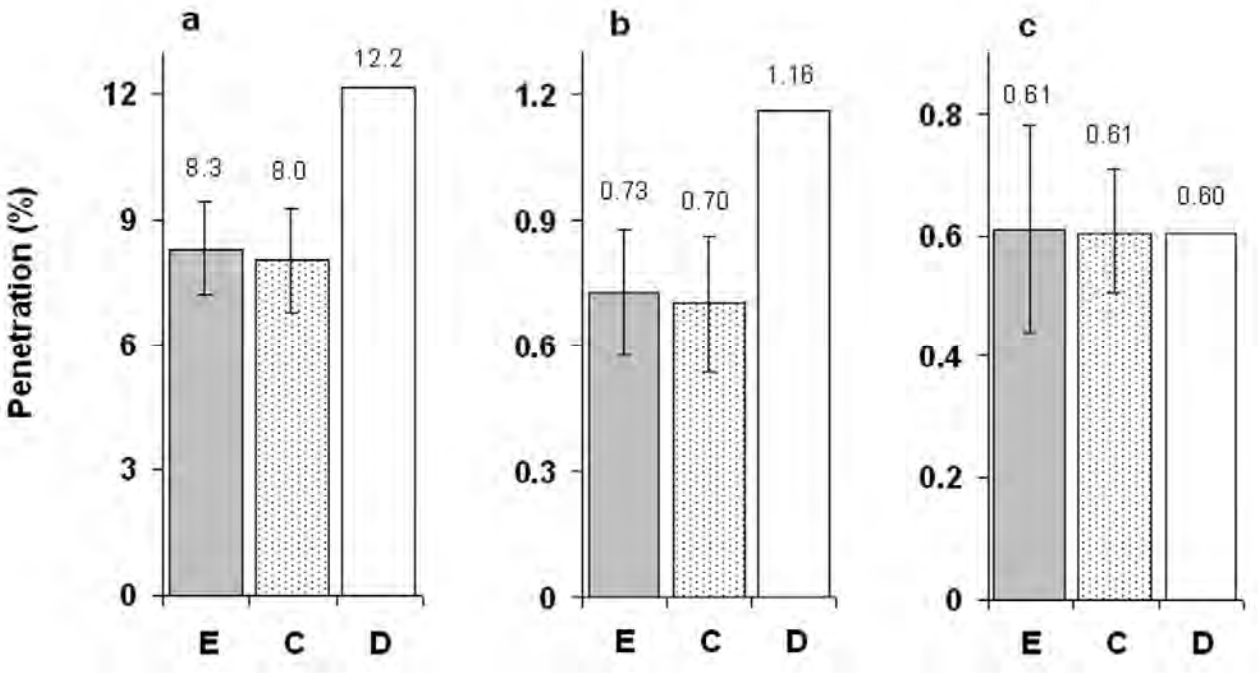

Figure 5-4. Particle penetration (±1 SD) for electrosprayed MS2 (E), Collison nebulizer MS2 (C), and electrosprayed dextrose (D) for each filter type: (a) surgical mask swatch; (b) N95 swatch; and (c) N100 swatch.

APPENDIX A: PEER REVIEWED PUBLICATIONS

APPENDIX A1

Eninger RM, Takeshi H, Reponen T, McKay R, Grinshpun SA (2008). What Does Respirator Certification Tell Us About Filtration of Ultrafine Particles? *J Occup Environ Hyg*; 5:286-295.

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What Does Respirator Certification Tell Us About Filtration of Ultrafine Particles?

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What Does Respirator Certification Tell Us About Filtration of Ultrafine Particles?

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Recent interest in exposures to ultrafine particles (less than 100 nm) in both environmental and occupational settings led the authors to question whether the protocols used to certify respirator filters provide adequate attention to ultrafine aerosols. The authors reviewed the particle size distribution of challenge aerosols and evaluated the aerosol measurement method currently employed in the National Institute for Occupational Safety and Health (NIOSH) particulate respirator certification protocol for its ability to measure the contribution of ultrafine particles to filter penetration. Also considered were the differences between mechanical and electrically charged (electret) filters in light of the most penetrating particle size. It was found that the sodium chloride (NaCl) and dioctylphthalate (DOP) aerosols currently used in respirator certification tests contain a significant fraction of particles in the ultrafine region. However, the photometric method deployed in the certification test is not capable of adequately measuring light scatter of particles below approximately 100 nm in diameter. Specifically, 68% (by count) and 8% (by mass) of the challenge NaCl aerosol particles and 10% (by count) and 0.3% (by mass) of the DOP particles below 100 nm do not significantly contribute to the filter penetration measurement. In addition, the most penetrating particle size for electret filters likely occurs at 100 nm or less under test conditions similar to those used in filter certification. The authors conclude, therefore, that the existing NIOSH certification protocol may not represent a worst-case assessment for electret filters because it has limited ability to determine the contribution of ultrafine aerosols, which include the most penetrating particle size for electret filters. Possible strategies to assess ultrafine particle penetration in the certification protocol are discussed.

Keywords certification, filtration, particle, respirator, ultrafine

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INTRODUCTION

Particulate-filtering respirators marketed in the United States are subjected to performance certification prior to becoming available commercially. Certification ensures that respirators meet prescribed performance criteria intended to ensure a minimum level of user protection. Certification also results in an explicit stratification of respirator types and classes that aid the health and safety professional in selecting a level of protection appropriate for a specific hazard.

U.S. government approval of respirators began in 1919 when the Bureau of Mines promulgated Approval Schedule 13 for self-contained breathing apparatuses.[1] Approval requirements for other respirator types followed, and certification requirements for particulate-filtering respirators were promulgated in 1934. With several modifications, the Bureau of Mines requirements eventually became the core respirator certification tests adopted by the newly formed National Institute for Occupational Safety and Health (NIOSH) in 1972.[2]

Currently, NIOSH certifies respirators in accordance with Title 42 of the U.S. Code of Federal Regulations, section 84 (42 CFR 84).[3] The regulations, adopted in current form in 1995, prescribe minimum performance requirements for respirator components and systems. Filtration efficiency of air-purifying particulate filters, the focus of this article, is certified under 42 CFR 84.181, Non-Powered Air-Purifying Particulate Filter Efficiency Level Determination. Respirators are certified in one of nine classes based on three levels of filtration efficiency and three levels of resistance to filter degradation (Table I).

Respirator filtration efficiency is tested and certified for 95%, 99%, or 99.97% removal of challenge aerosol particles. These respirators are respectively labeled as 95, 99, or 100 class efficiency. Filter series is categorized as N, R, or P based on the type of aerosol used for testing. N-type filters are intended to protect workers from solid particulates and are tested against a mildly degrading sodium chloride (NaCl) aerosol; R-type filters demonstrate resistance to liquid particulates and are tested against a more highly degrading dioctylphthalate (DOP) oil aerosol; and P-type filters are highly resistant to degradation and are tested against DOP until filter efficiency is at its lowest level.[3]

Because respirator users encounter a wide variety of aerosols under varying conditions, respirator testing is a combination of "worst-case" and "very severe" conditions.[3]

TABLE I. Nonpowered Particulate Air-Purifying Respirator Classification (summary)

Respirator	Minimum Filtration Efficiency (%)	Challenge Aerosol	Maximum Filter Loading	Usage Limitation	Certification Preconditioning[A]	Certification Flow Rate
N95	95	NaCl	200 mg	Non-oil aerosols only	Y	
R95	95	DOP	200 mg	8-hr/one workshift[B]	N	
P95	95	DOP	Lowest efficiency	Per user instructions[C]	N	
N99	99	NaCl	200 mg	Non-oil aerosols only	Y	
R99	99	DOP	200 mg	8-hr/one workshift[B]	N	85 L/min
P99	99	DOP	Lowest efficiency	Per user instructions[C]	N	
N100	99.97	NaCl	200 mg	Non-oil aerosols only	Y	
R100	99.97	DOP	200 mg	8-hr/one workshift[B]	N	
P100	99.97	DOP	Lowest efficiency	Per user instructions[C]	N	

[A] N-series filters require preconditioning at 85% relative humidity and 38°C for 25 hr.
[B] In oil aerosol environment.
[C] P-series respirator filter service life recommendations are manufacturer specific.

Research has shown mild filtration degradation when N-type filters are stored in high relative humidity conditions.[4] Consequently, filters undergo preconditioning at 85% relative humidity and 38°C for 25 hr prior to testing.[3,4] The generated challenge aerosols are "charge-neutralized," which increases filter penetration when compared with charged aerosol particles.[3,5] The test aerosols are intended to be at (or near) the assumed most penetrating particle size of 0.3 μm in aerodynamic diameter.[3,6,7] Because the respirator wearer's breathing minute ventilation can alter filter efficiency, an airflow of 85 L/min (or 42.5 L/min for dual filter respirators) is used to represent a worker's inhalation at a high work rate.[3,7]

Respirator certification is intended to be a conservative test in order to ensure a minimum level of filtration in a wide variety of workplaces, with differing aerosol characteristics, environmental conditions, and workloads.[3] The current certification protocol, however, may not address the filter efficiency against ultrafine particles (with a diameter less than 0.1 micrometers = 100 nm), although this fraction is of special interest in environmental and occupational hygiene for several reasons.[8] Due to high surface area per unit mass, ultrafine particles often have significantly different biological activity compared with larger airborne particles of the same composition.[9] Patterns of respiratory deposition of ultrafine particles are not well characterized, and there are no accepted particle-size selective criteria for their monitoring.[8,10,11] Occupational sources of ultrafine particles are numerous. Some common sources involve combustion, such as diesel or aircraft exhaust, or welding fume generation.[8]

In addition, concern over appropriate protection against bioaerosols has increased in recent years. Microbial fragments have been observed in the ultrafine size range, and it is hypothesized that viruses can be aerosolized in the ultrafine size range as droplet nuclei or single virions.[12,13] Lastly, recent developments in nanotechnology have resulted in a considerable interest in the health and safety aspects of engineered nanoparticles. These materials, used in a wide variety of commercial applications and products, include particulate materials <100 nm in size that are engineered and manufactured with specific or unique properties. Risk assessment and management of engineered nanoparticles is still in infancy and guidance on exposure assessment and respiratory protection is limited.[14,15]

Lack of practical and cost-effective technologies to evaluate exposures, as well as uncertainty in the risks posed by ultrafine and nanoparticles, has spurred an increasing awareness in the occupational health community and a corresponding demand for systematic research and guidance. The respirator certification protocols were developed and adopted prior to ultrafine and nanoparticle risk management possessed the importance it does today. Therefore, the purpose of this study was to evaluate the existing NIOSH respirator certification protocol from the perspective of its ability to provide users information on filtration in the ultrafine particle size range.

REVIEW OF NIOSH PROTOCOL FOR TESTING FILTRATION AND METHODOLOGY FOR EVALUATION OF THIS PROTOCOL

Overview

The parameters of a respirator filtration test critically affect the findings on the respirator performance and, consequently, the practical implications of the test outcome. Four primary determinants of aerosol filtration are (1) challenge aerosol characteristics, (2) the respirator filter characteristics, (3) the aerosol measurement method, and (4) the test conditions (Figure 1). Challenge aerosol characteristics include (but are not limited to) the physical state and density of particles, the particle size distribution, and electrical charges. Respirator filter characteristics include the filter substrate, surface area, thickness, fiber diameter, surface density, and fiber electrical charge (for electret filters). Aerosol measurements are concerned generally with the particle count, surface area, mass (or related characteristic such as light scatter); the measurement method is based on a specific principle, such as gravimetry

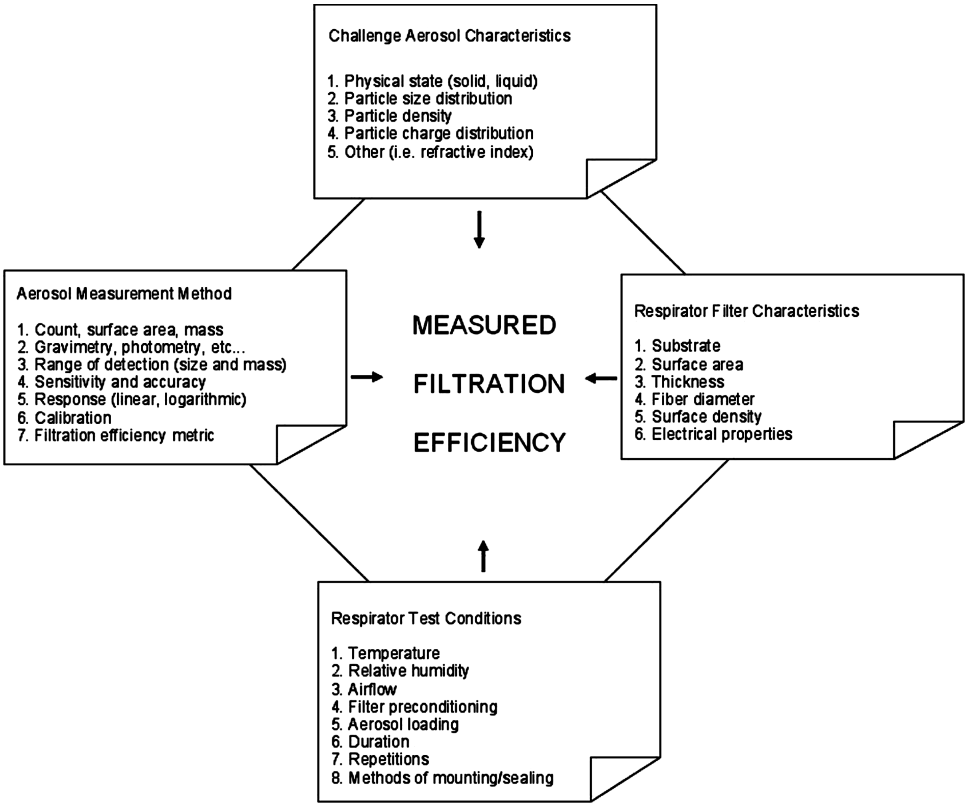

FIGURE 1. Factors influencing respirator filter test results

or photometry, and characterized by the limit of detection and other factors.

Test conditions are characterized by the temperature, relative humidity, flow, filter preconditioning, loading, test duration, number of test replicates, and procedures chosen for mounting/sealing a filter in a test chamber. Three of these parameters—challenge aerosol particle size distribution, filter electret properties, and challenge aerosol measurement method—were evaluated in this study to define the lower boundary of detectable particle size associated with the NIOSH filtration certification test.

The methods to perform this evaluation are summarized here. The physical characteristics of challenge aerosols used in the NIOSH respirator certification protocol were reviewed. Next, the size-fractioned light-scatter of the NIOSH test aerosols was modeled, and aerosol measurement methods for determining filtration efficiency were evaluated with respect to their ability to detect the contribution of all particle sizes present. Finally, strengths and shortcomings of the existing certification aerosol detection methods were reviewed in light of published findings about the most penetrating particle size (MPPS) for electret filters.

Challenge Aerosols

First, an ideal aerosol for use in testing respirator filtration should be safe to use, easy to generate, measure, maintain a stable challenge concentration, and replicate at different laboratories. Second, its penetration through respirator filters should represent a or "very severe" or "worst-case scenario" relative to the expected workplace aerosol contaminants. Third, it should be as degrading or more degrading to a filter material than workplace aerosols. No single challenge aerosol fulfills all of these requirements, and filter testing for nonworkplace contaminants and environments (i.e., military applications) may need to differ.

The aerosol used by NIOSH to evaluate respirators for use against solid particles is sodium chloride (NaCl).[3] The test aerosol is required to have a 75 ± 20 nm count median diameter (CMD) and a geometric standard deviation (GSD) of less than or equal to 1.86. Based on a density of 2.13, it has a mass median aerodynamic diameter (MMAD) of 347 nm.[16] The aerosol that NIOSH uses to evaluate respirators against liquid particles is dioctyl phthalate (DOP).[3] This oil-based aerosol was chosen for its degrading properties and is required to have a CMD of 165 ± 20 nm and a GSD < 1.6. Based on a density of 0.986, its MMAD is 356 nm.[17] The characteristics of both challenge aerosols are summarized in Table II and Figure 2.

Logarithmic distributions theoretically have no upper limit; the authors assumed an upper bound of 1 μm. In practice, this is a reasonable estimate of the largest particle sizes observed in the certification tests. In the literature, the NIOSH challenge aerosol is often referred to as "0.3 μm in size," which, technically means the mass median aerodynamic diameter discussed here. The above indicated aerodynamic diameter

TABLE II. NIOSH Challenge Aerosol Characteristics for Particulate Respirator Filtration

Challenge Aerosol	Density	CMD[A]	GSD	MMD[B]	MMAD
Sodium Chloride, NaCl	2.13	75 ± 20 nm	≤1.86	238 nm	347 nm
	% count distribution:[C]		% mass distribution:[C]		
	±1SD (68%)	40–140 nm	±1 SD (68%)	128–443 nm	
	±2SD (95%)	22–252 nm	±2 SD (95%)	70–732 nm	
Dioctylphthalate, DOP	0.986	165 ± 20 nm	≤1.60	359 nm	356 nm
	% count distribution:[C]		% mass distribution:[C]		
	±1SD (68%)	116–295 nm	±1SD (68%)	224–560 nm	
	±2SD (95%)	73–464 nm	±2SD (95%)	142–821 nm	

[A] Per 42 CFR 84.181(g), the challenge aerosol CMD must be within ± 20 nm.
[B] Count and mass distributions differ slightly from those predicted by the logarithmic function due to assumed 1 μm upper particle size.
[C] Calculated using Hatch-Choate equations.[6]

was selected based on a most penetrating particle size (MPPS) predicted by single-fiber filtration theory of mechanical filters, which is applied to respirator filters undergoing the NIOSH testing program.[3,6]

The charges carried by the challenge aerosol particles influence filter penetration. The NIOSH challenge aerosols are equilibrated to a bipolar Boltzmann charge distribution, which results in zero net charge. This is commonly referred to as a "charge-neutralized" aerosol. Because individual particles of a charge-neutralized aerosol may carry a positive or negative charge, this is an example of a "very severe" rather than a "worst-case" test condition. A worst-case condition, although not as applicable to workplace aerosols, occurs when both individual particles and the aggregate aerosol possess no net charge.[5,18]

Aerosol Measurement Method

An ideal aerosol measurement method for testing filters should be rapid, accurate and reproducible, maintain calibration, and cover an appropriate particle size range that includes the most penetrating particle size (MPPS) for all tested filter materials. It seems that none of the currently available measurement methods meet all the above criteria.

The NIOSH testing protocol utilizes two forward-light scattering photometers to simultaneously measure aerosol concentrations before (upstream) and after (downstream) the respirator. Photometers measure the amount of light scattered by an assemblage of aerosol particles, which for certain particle sizes is proportional to aerosol mass.[19] For a given wavelength of incident light (λ), scattering angle, and particle index of refraction, the flux of scattered light by an assemblage of particles (R) is proportional to concentration and depends on the particle size distribution according to the following relationship:[19]

$$R = c_n \int_0^\infty f(d_p) P_\lambda(d_p) d(d_p) \qquad \textbf{(1)}$$

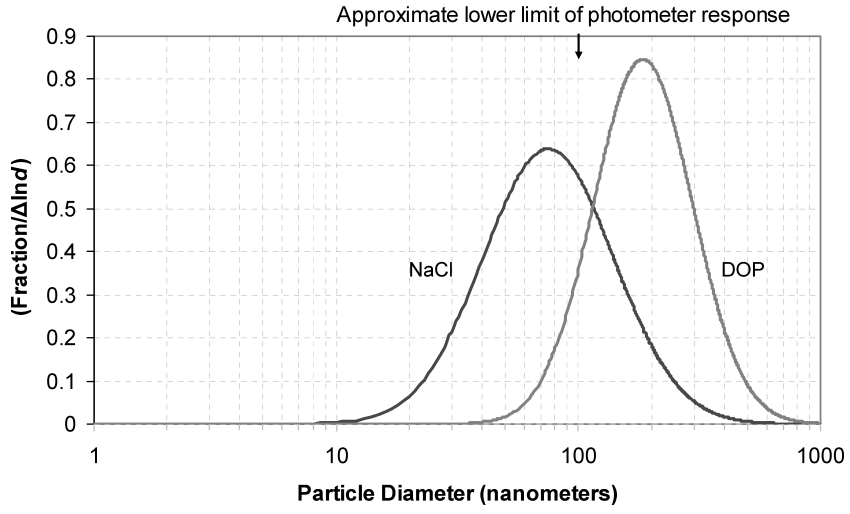

FIGURE 2. Challenge aerosol particle size distributions (by count) and photometer limit of detection

Here, c_n is the particle number concentration, $f(d_p)$ is the particle size distribution probability density function, and P_λ is the single-particle flux of scattered light. The NIOSH filter testing protocols use a specific incident light wavelength, particle indices of refraction, and scattering angle, which are the same for upstream and downstream measurements. Therefore, the effect of particle size on the measurement results is the focus.

The certification testing deploys a TSI model 8130 Automated Filter Tester (TSI, Inc., St. Paul, Minn.) that embeds two forward laser-scattering photometers for upstream and downstream particle concentration measurements. Scatter from the 780 nm wavelength laser light is measured at 45° of incidence. For particle physical diameters less than approximately half the incident light wavelength ($^1/_2\lambda$), scatter is proportional to d_p^6 and increases monotonically with increasing particle size.[19] For the NIOSH challenge aerosol, this includes particles up to ~380 nm in physical diameter. For aerosol particles larger than 380 nm ($>^1/_2\lambda$), scatter is overestimated by this relationship, and more sophisticated methods are required.[19] As the aerosol concentrations upstream and downstream of the filter are indicated by the photometer output voltages, percent filter penetration is calculated as the ratio of these concentrations, corrected for a zero background, multiplied by 100:

$$P = \frac{C_{down}}{C_{up}} \times 100\% \qquad \textbf{(2)}$$

Note that P, which represents penetration in this equation, is separate and distinct from the earlier defined P_λ.

Photometry is extremely useful, as it provides a rapid way to estimate upstream and downstream aerosol concentrations when testing the filter performance. However, there are practical limits of photometry associated with the ultrafine particle size range. Generally, 100 nm is considered the smallest particle diameter that measurably contributes to a photometer signal.[19,20] Additionally, 100 nm is the lower limit of particle size detected in the device used in the NIOSH test protocol.[21] This limit is imposed by a combination of background light-scatter from the fluid medium, light sensitivity limits due to the required detection range of photometers, and limits in the photometer light-sensing optics. Figure 2 superimposes this lower limit of detection on the test aerosol particle size distributions. From this figure, it is apparent that most of the NaCl particles and a considerable portion of the DOP particles (by size) may not contribute to the photometric concentrations used to certify respirator filtration.

To determine the contribution to light scatter available for photometer detection by size fraction, the authors modeled the single particle light scatter, P_λ, for each test aerosol based on its upstream particle size distribution and physical characteristics using MiePlot version 3.5.01 (Philip Laven, Geneva). This software allows for modeling of various user-defined aspects of optical and electromagnetic scattering by particles in accordance with Mie theory.[22] The modeling parameters included challenge aerosol refractive index (NaCl: 1.544, DOP 1.485), medium refractive index (1.0), light scatter angle (45°), and incident light wavelength of 780 nm with perpendicular polarization.[16,17] The model output was the relative intensity of scattered light at 45°.

For the model, the scattering particles were assumed to be homogenous spheres. This very nearly is the case for the DOP aerosol particles but not for the NaCl particles, which are a face-centered cubic crystal structure. Previous studies have shown that Mie scattering provides a reasonable estimate of light scatter for nonspherical particles in certain circumstances. Perry et al.[23] studied light scatter of aerosolized salt particles up to 1 μm in diameter and observed that light scatter in the forward direction was relatively independent of shape for particles with a size parameter up to 3. For the NIOSH sodium chloride challenge aerosol, this observation would apply to particles smaller than 745 nm.

More recent work by Chamaillard et al.[24] compared the differences in modeled light scatter estimates for sea salt crystal aerosol particles from ~100 nm to 2 μm in size using Mie theory and discrete dipole approximation (DDA).[24] Discrete dipole approximation uses a volume integral equation to describe the interaction of electromagnetic waves and objects and is applicable to estimating scatter from nonspherical particles. Chamaillard et al. observed little or no difference in scatter between the two models for particles smaller than 300 nm. They also reported that Mie theory underestimated particle scatter by ~10% for salt particles greater than 800 nm. Thus, the authors' assumption of spherical NaCl particles seems reasonable for the purpose of this investigation and did not have essential impact on their observations.

After multiplying the modeled single-particle flux of scattered light (P_λ) with the aerosol particle size density function, the size-fractioned contribution to light scatter was obtained for each challenge aerosol. These values were then summed over the challenge aerosol particle size distributions up to one micrometer:

$$R_{cum} = \sum_{0\ \text{nm}}^{1000\ \text{nm}} f(d_p) P_\lambda(d_p) \qquad \textbf{(3)}$$

The above cumulative scatter function was plotted with the challenge aerosol count and mass cumulative functions to provide a side-by-side comparison of cumulative count, mass, and light-scattering response for the two challenge aerosols.

RESULTS AND DISCUSSION

The results of the authors' analysis are shown in Figures 3a and 3b. In addition, Table III presents a summary of the size-fractioned contributions to count, mass and scatter for each NIOSH challenge aerosol.

The figures allow determining the count or mass of the challenge aerosols relative to light scatter. It is seen from Figure 3a and Table III that NaCl particles smaller than 100 nm comprise 68% by count and about 8% by mass but essentially

TABLE III. Percent Contribution by Size for Two Challenge Aerosols

Particle Size Range (nm)	NaCl Test Aerosol (%)[A]			DOP Test Aerosol (%)[A]		
	Count	Mass	Scatter	Count	Mass	Scatter
0–100	68	8	0.6[B]	10	0.3	<0.01[B]
100–200	26	31	8	47	11	2
200–300	4	26	20	28	25	12
300–400	1	15	25	10	24	24
400–500	0.2	9	23	3	17	28
500–600	0.07	5	13	1	10	19
600–700	0.02	3	7	0.4	6	10
700–800	<0.01	2	3	0.1	3	5
800–900	<0.01	1	0.7	0.05	2	1
900–1000	<0.01	0.6	0.3	0.02	1	0.4

[A] Columns may not add to 100% due to rounding error.
[B] Scatter values for 0–100 nm are theoretical; scatter from ultrafine particles is poorly detected by photometers.

do not contribute to the light scatter available for photometer detection. On the other hand, the largest 0.3% of particles by count and largest 21% by mass provide half the light scatter. Approximately 80% of the light scattering is provided by particles 270 nm and larger.

For DOP, Figure 3b and Table III show a similar result: ultrafine particles of DOP, which make up 10% of the count, and about 0.3% of the mass have essentially no contribution to light scatter, whereas the largest 3% of particles by count and largest 30% by mass provide half the light scatter.

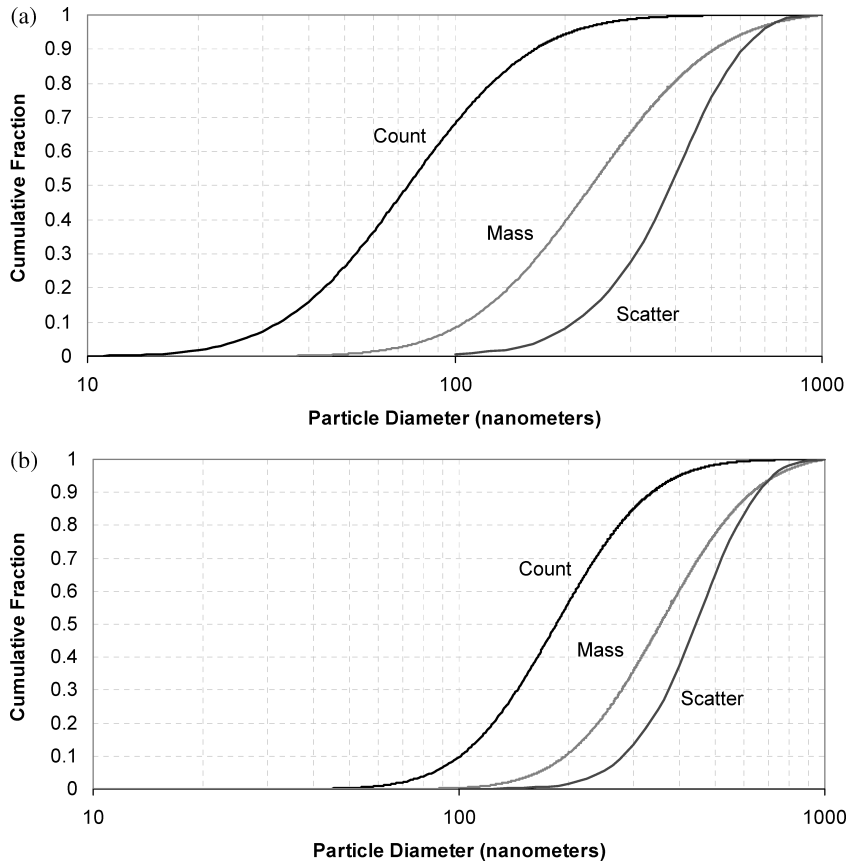

FIGURE 3. (a) NaCl challenge aerosol cumulative fractions: count, mass, light scatter; (b) DOP challenge aerosol cumulative fractions: count, mass, light scatter

Approximately 80% of the light scattering is provided by DOP particles 350 nm and larger.

The analysis indicates that the NIOSH certification test protocol (as directed by 42 CFR 84.181) effectively does not measure the contribution to filter penetration made by particles in the ultrafine size range. Particles less than 100 nm in size are present in both challenge aerosols; however, these particles essentially do not contribute to the photometer signal used for measuring the aerosol concentration. Therefore, the certification protocol—as it is now administered—has limited ability to provide users with information on the respirator filter efficiency against ultrafine particles. This finding seems to be of particular importance due to the uncertainties of health effects associated with environmental ultrafine aerosol particles and engineered nanoparticles (<100 nm). Workplace risk management of potential occupational hazards from engineered nanoparticles is an area of ongoing research. NIOSH has identified respiratory protection as a critical topic area with respect to knowledge gaps about nanotechnology and occupational health.[25]

The existing certification test may not assess filtration efficiency for the particle sizes that represent the worst-case scenario in terms of collection by respirator filters with electret properties (i.e., exhibit the highest penetration). The MPPS for a specific filter system is determined by several factors, including airflow, fiber charge density, and aerosol particle charge distribution.[6,7,18,26,27] The NIOSH presumption of a most penetrating size of approximately 300 nm (MMAD) may not hold for electret filters under NIOSH test conditions. A summary of evidence in support of this is shown in Table IV and discussed here.

It has been shown with NaCl challenge aerosol that peak aerosol particle penetration through polypropylene electret filter material may occur at particle diameters much less than 300 nm. The MPPS, which has been shown to decrease with increasing filter face velocity, consistently appears to be less than 100 nm for aerosols in uncharged and Boltzmann charged conditions. Baumgartner and Loffler[28] evaluated two types of electret filters against 20–250 nm NaCl particles in charge equilibrium. For split-type fibrous filters, peak penetrations occurred at approximately 30 nm while they were observed at ~70–80 nm for the electrostatically spun filter. Lathrache and Fissan[29] tested three types of commercially available electret filters against the Boltzmann-charge-equilibrated NaCl and diethylhexyl-sebacate oil (DES) aerosols with a particle diameter of 20 nm to 1 μm at varying face velocities. Penetration was observed to have a bimodal dependence on particle size, with a MPPS below 100 nm in six of eight test conditions. Kanaoka et al.[26] evaluated a rectangular fiber electret filter against NaCl aerosol particles ranging from 20 to 400 nm in various charge states. The observed MPPS of uncharged and equilibrium-charged particles was less than 100 nm. Oh et al.[30] performed a numerical simulation of single-fiber filtration efficiency of a unipolar charged fiber against particles smaller than 1 μm and compared results with laboratory measurements. Using a semi-empirical approach, the authors incorporated simulation of filter deposition by mechanical and electrical means. The model, being in agreement with experimental data, predicted an MPPS of ~85 nm.

In addition to testing filter materials, evaluations have been conducted specifically with respirators utilizing electret filters. Brosseau et al.[31] evaluated the collection efficiency of 10 electret dust/mist filters against latex spheres 102 nm to 2 μm in size at a Boltzmann charge distribution. Peak penetration for all filters was observed to occur at the smallest test particle size of 102 nm; results suggested an MPPS equal to or less than 102 nm. Stevens and Moyer[7] tested various types of air-purifying respirator filters against NaCl and DOP aerosols in Boltzmann charge distribution with a particle size ranging from 30 to 300 nm at varying face velocities. Peak penetration was observed to be below 100 nm for all filter types, except for the tested high-efficiency (HE) filters—a precursor designation to the current N or P-100 type filters. Fardi and Liu[32] evaluated several models of filtering facepiece respirators (FFR) against charge-neutralized NaCl and DOP aerosols from 35 nm to 4 μm in size. Those respirators with electret properties showed peak penetrations at approximately 100 nm, whereas those without were between 300 and 400 nm. More recently, Martin and Moyer[27] studied the size-fractioned filtration efficiency of various FFRs against both NIOSH certification test aerosols before and after removing the filter electret charge. They observed that removing the electret charge resulted in significantly higher penetration and a shift in the MPPS from 50–100 nm to >250 nm.

The authors' team at the University of Cincinnati studied the size-fractioned penetration of aerosolized NaCl particles and MS2 bacteriophage virions with a Boltzmann charge distribution through N95 filtering facepiece respirators and observed an MPPS <100 nm.[33,34] Richardson et al.[35] observed similar results when testing N95 FFRs and cartridges under varying constant and cyclic flow conditions using neutralized NaCl, DOP, and MS2 bacteriophage aerosols. Lastly, NIOSH researchers recently published a study of size-fractioned N95 FFR penetration using Boltzmann-charged NaCl aerosol 20 to 400 nm in size. They consistently observed an MPPS of approximately 40 nm.[36] According to conventional mechanical filtration theory,[6] a NaCl particle with a physical diameter of ~65 nm equates to an MMAD of ~300 nm, which is the currently accepted MPPS. It is important to note that for electret filters, the aerosol filtration in the ultrafine size range is governed by the physical particle diameter rather than the aerodynamic diameter. This is supported by the observation that aerosols at or near unit density, including paraffin, DOP and DES oils, polystyrene latex (PSL), and MS2 bacteriophage virus, also show an MPPS less than 100 nm for electret filters, as discussed above.

There are two primary findings of this study. First, the authors' analysis shows limitation of the 42 CFR 84.181 respirator certification protocol as it is currently implemented, which does not assess filtration of ultrafine particles. The particles <100 nm essentially do not contribute to the light

TABLE IV. Summary of Studies: Electret Filter Penetration and Most-Penetrating Particle Size

Study	Filter/Respirator Properties[A] Material	α	d_f (μm)	L (mm)	q or σ	Challenge Aerosol (Charge Condition)	V_f^B (cm/sec)	MPPS[C] (nm)	Comments
Baumgartner and Loffler[28]	Split fiber Spun fiber		$\rho_{sf} = 250$ g/m^2 $\rho_{sf} = 30$ g/m^2		Not provided	NaCl, 20–250 nm (Boltzmann)	10	<100	Filter properties not provided
Lathrace and Fissan[29]	Split fiber Spun fiber	0.042 0.035	30 5.2	5 1	2e-4–5e-4 C/m^2 8e-3–0.2 nC/m	NaCl, 20–189 nm DES, 140 nm–1 μm (Boltzmann)	2–30	<100	MPPS > 100 nm in two of six test conditions
Kanaoka et al.[26]	PP	0.031 0.075	~24*	4 5	34.2 nC/m	NaCl, 20–400 nm, (Uncharged, Boltzmann)	5–200	<50	*Rectangular fibers
Stevens and Moyer[7]	Commercially available dust/mist, paint/lacquer/enamel/mist, dust/fume/mist, and high-efficiency (HE) respirator filters; Filter materials: wool, wool resin, electrostatic felt, felt resin, fiberglass					NaCl, 30–240 nm; DOP, 30–300 nm (Boltzmann)	Q = 16–85 L/min	55–120	MPPS of all tested HE filters 90–175 nm
Brosseau et al.[31]	Ten commercially available dust/mist respirators filters: nine were combined resin-impregnated wool and PP felt, one was PP					Latex spheres, 102 nm–2.02 μm (Boltzmann)	55.3–74.8	≤102	Q = 2.7 L/min; filter surface area range: 36.1–48.8 cm^2
Fardi and Liu[32]	One mechanical dust/mist and two electret dust/mist or dust/mist/fume respirator filters					NaCl, DOP 35 nm–4 μm (Boltzmann)	Q = 16, 32, 45 L/min	~100	Face velocity or filter surface area not provided
Martin and Moyer[27]	Six commercially available models of FFR: three N95, one N99, one R95, one P100					NaCl, DOP ~25–400 nm (Boltzmann)	Q = 85 L/min	50–100	Aerosols complied with 42 CFR 84.181; filter surface area not provided
Oh et al.[30]	Spun filter	0.04	9		1.44 nC/m	NaCl (Boltzmann)	10	~85	Numerical simulation; good agreement with Baumgartner and Loffler
Balazy et al.[33]	PP FFR PP FFR	0.069 0.091	7.84 7.19	1.77 0.35	13–14 nC/m* Not provided	NaCl, ~10–600 nm (Boltzmann)	4.5, 12.9 3.7, 10.6	40–50	Two models of N95 FFR; *q was estimated
Balazy et al.[34]	Same as Balazy[33]					MS2 bacteriophage, ~30–80 nm (Boltzmann)	4.5, 12.9 3.7, 10.6	40–60	Same as Balazy et al.[33]
Richardson et al.[35]	Commercially available devices: two N95 FFR, two P100 FFR, two N95 cartridges, two P100 cartridges					NaCl, DOP 20 nm–3.02 μm; PSL .7–2.9 μm (Boltzmann)	0.7–60 See comments	50–100	MPPS for P100 > 100 nm; cyclic (40–135 L/min) and constant flow (42.5–180 L/min) test conditions
Rengasamy et al.[36]	Five commercially available models of N95 FFR					NaCl 20–400 nm (Boltzmann)	Q = 85 L/min	40	Filter surface area not provided

[A] PP = polypropylene; α = filter packing density; d_f = fiber diameter; L = thickness; q (nC/m) or σ (C/m^2) = charge density; ρ_{sf} = surface density.
[B] V_f = filter face velocity; provided where given or can be calculated from data provided in study; Q = volumetric flow rate.
[C] MPPS = most penetrating particle size.

scatter available for photometer detection and those between 100 and 200 nm contribute rather little, so that the photometer-measured filtration efficiency is not determined for the above fractions. Second, based on a review of the existing literature, the most penetrating particles for electret filters appear to belong to the ultrafine size fraction when challenged with an aerosol with a Boltzmann charge distribution. The contribution to light scatter determined in our analysis is dominated by particles larger than 200 nm in both test aerosols (NaCl and DOP) while representing 6% of the NaCl and 43% DOP particles by count. According to Martin and Moyer, quantifying filter penetration by count methods will "always be equal to or exceed a photometrically determined value."[27] The results of this study provide one explanation for this: light scatter is dominated by the larger particle sizes in the test aerosols, and those particles most likely to penetrate the filter are not measured by photometric means for electret filters.

The particle size fraction <200 nm, for which the limitations of the existing respirator testing protocol were demonstrated, can represent various workplace aerosols, including welding fume, diesel particles, viruses or viral droplet nuclei, bioaerosol fragments, and engineered nanoparticles. The filter certification via photometry seems to be most appropriate for the respirators used against aerosol hazards with mass-based exposure metrics. Research has suggested that as particle size decreases, particle surface area or count becomes a better predictor of health effects than aerosol mass.[14] Using photometry for filter testing implies a greater toxicological importance to protect users from particles with greater mass. The concept that "more mass means greater health effects" is no longer axiomatic within industrial hygiene practice. Given the wide range of occupational aerosols, it may be that no single aerosol detection method can serve all needs. Particle count may be more appropriate than photometric methods when testing respirator filters for use against certain hazards. This approach would be able to detect and enumerate particles smaller than 100–200 nm, and commercial technology for this does exist. To ensure a worst-case testing scenario, in terms of the ability to detect the most penetrating particle size, a count-based method of aerosol detection would appear to be preferable.

Estimating ultrafine particle penetration in conjunction with the existing respirator certification protocol may be possible using one of two strategies: (1) modeling that would require using proprietary filter specification data, or (2) additional data collection to establish a reliably predictive correlation between penetration above and below 100 nm. This last strategy is promising as shown recently by Rengasamy et al.[36] In their study, penetration of five N95 respirator filters using the NIOSH certification protocol was plotted against count-based penetration of 40 nm monodisperse particles and the relationship described using regression. They showed that (1) the relative performance of respirators was similar for particles above and below 100 nm among the respirators tested, and (2) a descriptive relationship to predict respirator performance below 100 nm using the existing NIOSH protocol data may be possible. However, significant additional study would be required to derive a reliably predictive relationship for filter cartridges and facepieces in each filter class. Consideration should be given to pursuing this approach further.

CONCLUSIONS

The physical characteristics of aerosols used in the current NIOSH respirator certification/testing protocol were reviewed. According to the protocol, filtration efficiency is determined by measuring the aerosol concentrations upstream and downstream of a filter using a forward light scattering photometer, which is capable of adequately measuring light scatter of particles significantly above 100 nm. The presently accepted protocol has limited ability to measure the contribution of smaller particles, especially in the ultrafine fraction (<100 nm). The latter includes the particles that have been shown to exhibit the highest penetration through electret filters under NIOSH test protocol conditions. Additionally, the information provided by the certification test does not allow evaluating how penetration varies based on particle size. It is concluded that while the NIOSH certification is effective at determining filtration efficiency against the majority of workplace aerosols, it is generally limited to providing respirator users performance data for particles greater than about 100 nm in physical diameter.

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APPENDIX A2

Eninger RM, Honda T, Adhikari A, Heinonen-Tanski H, Reponen T, Grinshpun SA (2008). Filter Performance of N99 and N95 Facepiece Respirators Against Viruses and Ultrafine Particles, *Ann. Occ. Hyg.* In Press, doi 10.1093/annhyg/men019.

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Filter Performance of N99 and N95 Facepiece Respirators Against Viruses and Ultrafine Particles

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The performance of three filtering facepiece respirators (two models of N99 and one N95) challenged with an inert aerosol (NaCl) and three virus aerosols (enterobacteriophages MS2 and T4 and *Bacillus subtilis* phage)—all with significant ultrafine components—was examined using a manikin-based protocol with respirators sealed on manikins. Three inhalation flow rates, 30, 85, and 150 l min^{-1}, were tested. The filter penetration and the quality factor were determined. Between-respirator and within-respirator comparisons of penetration values were performed. At the most penetrating particle size (MPPS), >3% of MS2 virions penetrated through filters of both N99 models at an inhalation flow rate of 85 l min^{-1}. Inhalation airflow had a significant effect upon particle penetration through the tested respirator filters. The filter quality factor was found suitable for making relative performance comparisons. The MPPS for challenge aerosols was <0.1 μm in electrical mobility diameter for all tested respirators. Mean particle penetration (by count) was significantly increased when the size fraction of <0.1 μm was included as compared to particles >0.1 μm. The filtration performance of the N95 respirator approached that of the two models of N99 over the range of particle sizes tested (∼0.02 to 0.5 μm). Filter penetration of the tested biological aerosols did not exceed that of inert NaCl aerosol. The results suggest that inert NaCl aerosols may generally be appropriate for modeling filter penetration of similarly sized virions.

Keywords: filter; penetration; respirator; ultrafine; virus

INTRODUCTION

Filtering facepiece respirators (FFRs) are protective devices used in numerous workplaces to reduce airborne particulate exposures. The US Bureau of Labor Statistics in partnership with the National Institute for Occupational Safety and Health (NIOSH) estimated in 2001 that over 200 000 private establishments in the US—totaling ∼1.9 million workers—had utilized disposable particulate FFRs in the 12 months prior to being surveyed (NIOSH, 2003).

Certification of respirator filtration under 42 CFR 84.181, *non-powered air-purifying particulate filter efficiency level determination*, is the portion of the regulations most salient to this paper (DHHS, 1995). Certification protocols test the filtration capability of respirators utilizing one of two polydisperse challenge aerosols: NaCl (for use against solid aerosols) or dioctylphthalate (DOP, for use against oil-based liquid aerosols). The challenge aerosols are intended to possess a mass median aerodynamic diameter of ∼0.3 μm, which is the approximate most penetrating particle size (MPPS) for filters as predicted by classic mechanical filtration theory (Hinds, 1999; Lee and Mukund, 2001). Certification conditions are supposed to represent the 'worst case' or 'very severe' scenario in testing filtration, i.e. a certified air-purifying FFR is intended to filter workplace aerosols as effectively (or more effectively) as it does when tested with the challenge aerosols under the NIOSH testing protocol.

Some limitations of the existing protocol for testing filters with electret properties challenged with ultrafine particles (<0.1 μm) have been discussed in our recent paper (Eninger *et al.*, 2008). Previous studies, reviewed in the above-cited paper have

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shown that for many electret filter materials, including those used to manufacture N-type respirator filters (N95 and N99), an uncharged or Boltzmann-charged (charge neutral) aerosol has the MPPS ≤ 0.1 μm in physical diameter. The shift in the MPPS from ∼0.3 to <0.1 μm has been attributed to the electret properties of the respirator filter (Lathrache and Fissan, 1986; Lathrache *et al.*, 1986), specifically to the polarization force affecting an electrically neutral particle and consequently changing the function of penetration versus particle size (Martin and Moyer, 2000; Balazy *et al.*, 2006a).

The conventional protocol utilizes two aerosol photometers—one before and one after the filter—to measure the particle penetration (DHHS, 1995; TSI, 2005, 2006). Photometer output signals are approximately proportional to aerosol mass and used to calculate filter penetration, *P*, as:

$$P = \frac{C_{\text{down}}}{C_{\text{up}}} \times 100\% \qquad (1)$$

where C_{down} is the challenge aerosol concentration downstream of the respirator filter, and C_{up} is the aerosol concentration upstream. However, photometry does not effectively detect the ultrafine aerosol fraction; it generally poorly detects the contribution of particles below ∼0.2 μm (Gebhart, 2001; Eninger *et al.*, 2008). As a challenge aerosol, NaCl has a significant fraction within the ultrafine size range: ∼68% of particles by count are <0.1 μm (while the DOP challenge has ∼10% of particles <0.1 μm by count). However, the amount of light scatter available for photometer detection contributed by the ultrafine fraction of both challenge aerosols is negligible, making the test conditions not fully adequate for the filter performance evaluation at MPPS <0.1 μm. Thus, the existing NIOSH certification protocol has a limitation in providing respirator users and occupational hygiene/health professionals with information on the ability of a respirator to filter ultrafine aerosols (Eninger *et al.*, 2008).

At the same time, the need in controlling ultrafine particle exposures has increased in recent years. Although the ultrafine component of occupational aerosols rarely contributes in a major way to exposure in mass terms, it can pose a significant exposure in terms of particle count or surface area (Donaldson *et al.*, 2001). Welding fume, diesel exhaust and some biological airborne particles are examples of aerosols containing a considerable ultrafine fraction (Vincent and Clement, 2000). An expanding source of ultrafine occupational exposures is the employment of engineered nanoparticles (Roco and Bainbridge, 2001; Maynard and Kuempel, 2005). Potential health effects of nanoparticle exposure are of an increasing interest (HSE, 2004; NIOSH, 2004, 2005a,b; Oberdörster *et al.*, 2007). Biological aerosols such as airborne viruses and fungal fragments often belong to the ultrafine fraction (Reponen *et al.*, 2001; Cho *et al.*, 2005). Both severe acute respiratory syndrome (SARS) and highly pathogenic influenza are caused by virions that can be <0.1 μm. Recent work by Morawska (2006) demonstrated that bioaerosol droplets can quickly dry in air to submicrometer and even ultrafine sizes and remain airborne for prolonged periods, thus representing a risk for infection.

Despite the need, there are limited data that can be utilized by health and safety practitioners for guidance in selecting respiratory protective devices for use with ultrafine aerosols, including airborne viruses. While some data on the filter performance of N95 respirators against nanoscale particles and MS2 virions have been recently published by this research group (Balazy *et al.*, 2006a,b), no similar performance information is available for N99 respirators (which are increasingly used in occupational environments, including healthcare settings). The purpose of this investigation was 2-fold: (i) to evaluate size-fractioned filter penetration of N99 FFRs against inert and biological ultrafine aerosols at a wide range of inhalation flow rates—from 30 to 150 l min^{-1} and (ii) to compare respirator filter penetration values within- and between-filter classes, model and challenge aerosol type (inert and biological). Thus, it is intended to serve as a follow-up of our previous work (Balazy *et al.*, 2006a,b) that examined N95 respirators at 30 and 85 l min^{-1}. The data collected in the present study provide respirator users with additional information for comparing filtration of N99 and N95 FFRs against ultrafine particles, including virions.

METHODS

Study design

The initial filter penetration through two N99 FFRs and one N95 FFR (selected for comparison) was evaluated at three flow rates (30, 85 and 150 l min^{-1}) against two types of challenge aerosol: inert and biological. The selected inert aerosol, NaCl of ∼20 to 500 nm in particle size, was utilized in testing all three respirators; the biological aerosols included MS2 bacteriophage virus (used to test all three respirators), *Bacillus subtilis* bacteriophage virus (N95 respirators) and enterobacteriophage virus type T4 (N95 respirators). Since most occupational exposures to ultrafine particles are low in mass terms and most FFRs are intended to be disposable respirators, the majority of their use (particularly in healthcare settings) will be in conditions with little or no particle loading. Therefore, this study examined only initial respirator filter performance and did not address filter loading. Additionally, this study did not evaluate the respirator face-seal leakage.

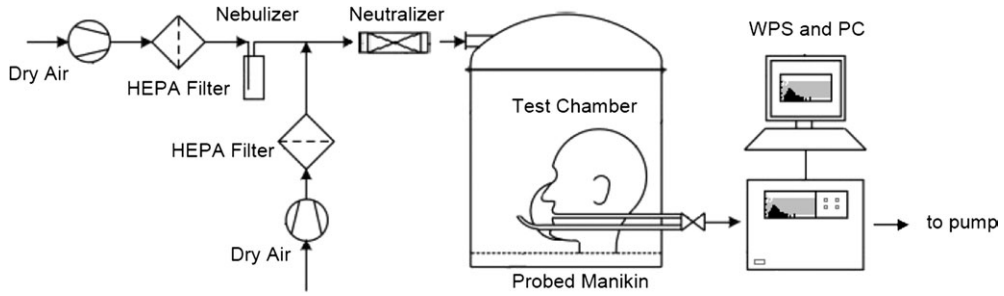

Fig. 1. Filter penetration test system. Diagram adapted from Balazy *et al.* (2006a,b).

Test system

The test system presented in Fig. 1 has been described in our earlier publications (Balazy *et al.*, 2006a,b). Challenge aerosol penetration through the respirators was evaluated using a manikin-based protocol. The respirator was sealed to a manikin face, leak tested and placed inside of a 0.096 m^3 test chamber. The challenge aerosol concentrations were measured upstream and downstream of the respirator facepiece. The aerosols were generated with a 6-jet Collison nebulizer (BGI Inc., Waltham, MA, USA), diluted and dried with clean air, charge equilibrated to a Boltzmann charge distribution using a Kr^{85} sealed source (Model 3054, TSI Inc., Minneapolis, MN, USA) and fed to the top of the test chamber. Constant inhalation flow was drawn through the probed manikin while size-fractioned particle counts from 20 to 500 nm in diameter were recorded outside and inside of the respirator facepiece using a Wide-range Particle Spectrometer (WPS, Model 1000 XP, MSP Corp., Shoreview, MN, USA) connected to the data acquisition system.

Respirator selection and test conditions

The two models of N99 and one model of N95 FFRs selected for this study are commonly used in industry and healthcare settings, based on the recommendations from the University of Cincinnati Occupational Pulmonary Services (Director, Roy McKay) that performs respirator fit testing and training for numerous industries in the US. The N95 respirator was of the same make and model as tested in our previous studies (Balazy *et al.*, 2006a,b). This model demonstrated relatively higher filtration of ultrafine particles when compared to other N95 models evaluated in our laboratory. Different manufacturers supplied the two N99 respirators (N99-A and N99-B).

The constant airflows (Q) of 30, 85 and 150 l min^{-1} were selected to represent different inhalation regimes. The first represents inhalation during low/moderate-intensity work. The second corresponds to a hard workload and is used by NIOSH for respirator filtration certification. The flow rate of 150 l min^{-1} was intended to represent an instantaneous peak inspiratory flow (PIF) during moderate to strenuous work (Harber *et al.*, 1984; Lafortuna *et al.*, 1984; Cassidy *et al.*, 2003). Consensus is not found in the literature for a representative occupational ventilation rate for PIF. However, the range of PIFs for the 95th percentile minute volume for occupational tasks is estimated to range between 182 and 295 l min^{-1} (Caretti *et al.*, 2004). Therefore, the choice of 150 l min^{-1} may underestimate a worst case PIF. Studying respirator filtration at higher inhalation flow rates is salient, at least, for two reasons. First, the rate established by the NIOSH protocol (85 l min^{-1}) may be exceeded during more strenuous occupational tasks. Second, modern FFR media relies upon electret properties for much of the overall filtration efficiency (Martin and Moyer, 2000; Caretti *et al.*, 2004). For ultrafine particles, the primary filter capture mechanisms are diffusion and electrostatic interaction, which are both strongly dependent upon respirator face velocity. This suggests the lowest collection efficiency (highest penetration) at the highest inhalation flow rate (Lathrache and Fissan, 1986; Lathrache *et al.*, 1986; Lee and Mukund, 2001).

Temperature and relative humidity were monitored during the tests using a DeltaTrak Thermo-Hygrometer (Model 13306, DeltaTRAK, Inc., Pleasanton, CA, USA). Relative humidity was maintained between 40 and 45% while temperature ranged from 23 to 26°C.

Selection and preparation of viruses

Three viruses were selected for use in filtration testing: enterobacteriophage types MS2 and T4 and bacteriophage *B. subtilis* SP01. These were chosen for their small particle sizes, low pathogenicity and ease of preparation and use. We intended to perform the tests with (i) the smallest virions as well as (ii) larger ones—of similar dimensions to those of the SARS coronavirus [~80 nm diameter (Goldsmith *et al.*, 2004)] and influenza A virus subtype H5N1 [~80 to 100 nm diameter (Madigan and Martinko, 2006a)]. MS2 has about the smallest size among viruses. T4 and *B. subtilis* bacteriophage are larger and

close to the SARS coronavirus and H5N1 by their volumetric equivalent sizes. It is acknowledged, however, that the latter two simulants are considerably different from the targets in terms of virion shape and aspect ratio, which may influence their filtration properties (Willeke *et al.*, 1996; Flagan, 2001; Rengasamy *et al.*, 2004). This is addressed further in the discussion.

MS2 is an icosahedral RNA bacteriophage which infects the male *Escherichia coli* bacteria (Valegård *et al.*, 1990). An icosahedron is a symmetric polyhedron with 20 triangular faces (Fig. 2); its shape is close to spherical (Madigan and Martinko, 2006a). A single MS2 virion has a physical diameter of ~28 nm (Valegård *et al.*, 1990; Madigan and Martinko, 2006b). T4 bacteriophage—which also infects many *E. coli* bacterial strains—is a double-stranded DNA bacteriophage with asymmetric icosahedral head, helical tail, endplate and tail fibers as shown in Fig. 2. A mature T4 virion is non-spherical. It is ~225 nm along its longest axis including the head (~85 × 100 nm), the tail (~25 × 100 nm) and the endplate (~50 × 25 nm) (Leiman *et al.*, 2003). *Bacillus subtilis* bacteriophage SP01 is also a double-stranded DNA bacteriophage with a structure similar to that of the T4 bacteriophage except with a roughly symmetrical icosahedral head (Hemphill and Whitely, 1975). A mature *B. subtilis* bacteriophage SP01 is typically 237 nm along its longest axis with a head and tail that measure 87 × 90 and 20 × 147 nm, respectively (Hemphill and Whitely, 1975).

MS2 (ATCC 15597-B1) and *B. subtilis* bacteriophage (ATCC 27370-B1) suspensions were prepared using lysis of host bacterial solutions—*E. coli* (ATCC 15597) and *B. subtilis* (ATCC 27370), respectively. This was followed by centrifugation to remove bacterial cells, their debris and particles from the medium then filtration with 0.4 μm sterile Millipore filter (Millipore Corp., Billerica, MA, USA). T4 bacteriophage suspensions were prepared from freeze-dried phage vial (ATCC 35060-B4) by adding 9 ml of Luria–Bertani broth followed by serial dilution. Suspensions of each phage for aerosol experiments were diluted to titre of 10^8–10^9 plaque-forming units per ml as determined by a modified plaque assay (ISO, 2000). ASTM reagent water purity type I ultrafiltered water was used for all suspensions (ASTM, 2006).

Filter penetration and quality factor

Particle concentrations were measured size selectively outside and inside the respirator filter when the inhalation flow was applied. The data were recorded in 24 size channels of the WPS' differential mobility analyzer ranging from 0.021 to 0.449 μm in particle electrical mobility diameter. Size-fractioned penetration was calculated using equation (1). Another metric of filter performance determined in this study was the filter quality factor, q_f, which incorporates airflow resistance (characterized by the pressure drop, Δp, in mmH_2O) and the particle penetration (P, %) (Hinds, 1999).

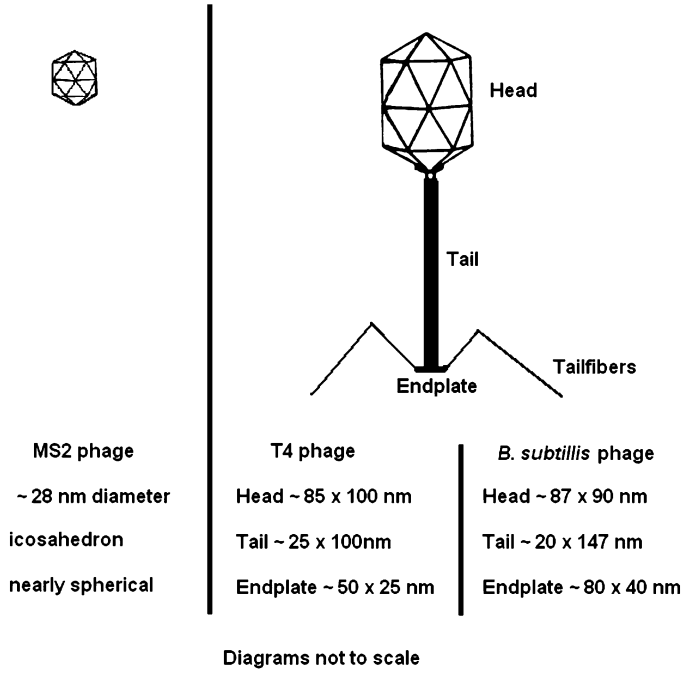

Fig. 2. Shape and dimensions of the bacteriophages used in this study.

$$q_f = \frac{\ln(1/P)}{\Delta p}. \quad (2)$$

An ideal respirator filter is characterized by low penetration and low pressure drop. Pressure drop across the filter media was measured at each inhalation flow rate using a magnehelic pressure gage (Dwyer Instruments, Inc., Michigan City, IN, USA).

Data analysis

The tests were replicated three times for each of the tested respirators and challenge aerosols. The mean, peak and standard deviation of the size-fractioned particle penetration were calculated for each combination of respirator, airflow rate and challenge aerosol. The pressure drop measured for a given respirator and airflow was applied to the corresponding size-fractioned penetration value to obtain the filter quality factor. Mean penetration (±1 SD) and filter quality factor were then plotted against electrical mobility particle diameter.

Between-respirator comparisons of the aerosol penetration were performed for two challenge aerosols: NaCl and MS2. The particle penetration through filters of all three respirator models was compared first using NaCl data and then using MS2 data. Within-respirator comparisons of penetration values for NaCl versus MS2 were also performed for all three tested respirator models. This database allowed us to compare the filter penetration of inert NaCl particles and airborne virions of the same particle sizes. Lastly, a within-respirator comparison with respect to penetration of NaCl, *B. subtilis* bacteriophage and T4 bacteriophage was performed for the N95 respirator. This also allowed comparing the filtration efficiency of inert particles to that of two biological aerosols. Overall, six comparative analyses were performed, as summarized below.

Between-respirator comparisons:

(1) NaCl challenge aerosol: compare penetration through N99-A, N99-B and N95 filters;
(2) MS2 challenge aerosol: compare penetration through N99-A, N99-B and N95 filters;

within-respirator comparisons:

(3) Model N99-A: compare penetration of NaCl and MS2;
(4) Model N99-B: compare penetration of NaCl and MS2;
(5) Model N95: compare penetration of NaCl and MS2 and
(6) Model N95: compare penetration of NaCl to that of phage *B. subtilis* and phage T4.

Comparisons 1 and 2 were performed using analysis of variance (ANOVA). Comparisons 3–5 were run using Student's *t*-test. Both ANOVA and Student's *t*-test with Bonferroni adjustment were utilized for Comparison 6. All tests were performed using Excel (Microsoft Corp., Redmond, WA, USA) at a significance level of 0.05.

RESULTS AND DISCUSSION

Aerosol penetration and filter quality factor

Aerosol penetration, pressure drop and quality factor for each test aerosol and inhalation flow rate are summarized in Table 1. For NaCl, the following specific particle sizes and ranges were selected for this summary table:

(i) 0.1 μm representing the approximate mobility sizes of phage *B. subtilis* and phage T4;
(ii) 0.3 μm representing the presently accepted MPPS;
(iii) 0.02–0.5 μm (integrated mean) representing overall penetration over the entire measured range of NaCl particle sizes and
(iv) 0.1–0.5 μm (integrated mean) representing the particle sizes which primarily contribute to filter efficiency determination using the NIOSH certification protocol.

For viruses, the following particle sizes were designated:

(i) 0.02–0.09 μm to represent the nominal virion size of MS2 and to include aggregates; the rationale for the selection of this particle size range is discussed in greater detail in Balazy *et al.* (2006b) (note that, resulting from slightly different WPS settings, the upper limit was modified—from 0.08 μm in Balazy *et al.* to 0.09 μm in this study) and
(ii) 0.1 μm to represent the approximate mobility sizes of phage *B. subtilis* and phage T4. A single WPS channel with a midpoint of 0.1 μm (range 0.094–0.11 μm) was used for the larger virions because a steep drop in the challenge aerosol particle size distribution beyond 0.1 μm suggested that aggregates, if present, did not considerably contribute to the total particle count.

NaCl challenge aerosol. Particle penetration increased with increasing airflow for all three respirators (Fig. 3) with the overall mean penetration at 150 l min^{-1} exceeding that at 30 l min^{-1} by an average factor of 7.9 (N99 Model A), 7.6 (N99 Model B) and 5.9 (N95). For all three respirators and inhalation airflows, the MPPS was <0.1 μm. Peak penetrations for N99 Model A were 10.2, 5.9 and 1.3%, respectively, for the high, medium and low flow rates; mean penetration at 85 l min^{-1} was 3.2% (for all particle sizes from 0.02 to 0.5 μm) and 1.6% (calculated

Table 1. Summary of aerosol penetration (P), pressure drop and quality factor (q_f) for three respirators

Aerosol: NaCl										
Respirator	Q (l min^{-1})	Pressure drop (mmH_2O)	$P_{0.1\mu m}$ (%)	$q_{f\ 0.1\ \mu m}$ (1 per mmH_2O)	$P_{0.3\ \mu m}$ (%)	$q_{f\ 0.3\ \mu m}$ (1 per mmH_2O)	$P_{0.02–0.5\ \mu m}$ (%)	$q_{f\ 0.02–0.5\ \mu m}$ (1 per mmH_2O)	$P_{0.1–0.5\ \mu m}$ (%)	$q_{f\ 0.1–0.5\ \mu m}$ (1 per mmH_2O)
N99 Model A	30	3.90 ± 0.20	0.66 ± 0.04	1.29 ± 0.06	0.35 ± 0.03	1.45 ± 0.10	0.75 ± 0.04	1.29 ± 0.06	0.43 ± 0.03	1.41 ± 0.08
	85	10.67 ± 0.58	2.92 ± 0.46	0.33 ± 0.03	0.99 ± 0.24	0.44 ± 0.05	3.20 ± 0.46	0.34 ± 0.03	1.60 ± 0.30	0.40 ± 0.04
	150	24.33 ± 2.08	5.14 ± 0.58	0.12 ± 0.01	2.07 ± 0.26	0.16 ± 0.01	5.93 ± 0.61	0.12 ± 0.01	3.13 ± 0.38	0.15 ± 0.01
N99 Model B	30	4.53 ± 0.15	0.74 ± 0.10	1.08 ± 0.06	0.44 ± 0.22	1.21 ±0.09	0.56 ± 0.11	1.20 ± 0.05	0.56 ± 0.18	1.17 ± 0.07
	85	13.00 ± 1.00	2.78 ± 0.34	0.28 ± 0.02	1.27 ± 0.50	0.34 ± 0.05	2.36 ± 0.20	0.31 ± 0.02	1.65 ± 0.14	0.32 ± 0.02
	150	24.67 ± 1.15	4.87 ± 0.94	0.12 ± 0.01	3.07 ± 1.96	0.15 ± 0.02	4.23 ± 1.27	0.13 ± 0.01	3.60 ± 1.53	0.14 ± 0.01
N95	30	2.70 ± 0.10	0.83 ± 0.20	1.78 ± 0.10	0.48 ± 0.14	1.99 ± 015	0.87 ± 0.21	1.80 ± 0.10	0.66 ± 0.21	1.89 ± 0.14
	85	7.57 ± 0.75	2.60 ± 0.51	0.49 ± 0.02	1.34 ± 0.44	0.58 ± 0.02	2.85 ± 0.44	0.49 ± 0.03	1.74 ± 0.41	0.54 ± 0.03
	150	15.83 ± 2.02	4.65 ± 0.48	0.20 ± 0.03	2.84 ± 0.38	0.23 ± 0.04	5.16 ± 0.35	0.19 ± 0.03	3.42 ± 0.46	0.26 ± 0.03

Aerosol: MS2			aerosol: *Bacillus subtilis* phage			aerosol: T4 phage		
Respirator	Q (l min^{-1})	$P_{0.02–0.09\ \mu m}$ (%)	Respirator	Q (l min^{-1})	$P_{0.1\ \mu m}$ (%)	Respirator	Q (l min^{-1})	$P_{0.1\ \mu m}$ (%)
N99 Model A	30	1.03 ± 0.55	N95	30	0.58 ± 0.22	N95	30	0.23 ± 0.01
	85	3.43 ± 0.86		85	1.90 ± 0.19		85	0.95 ± 0.11
	150	5.45 ± 0.35		150	3.81 ± 0.60		150	2.18 ± 0.37
N99 Model B	30	0.96 ± 0.12						
	85	3.28 ± 0.20						
	150	5.70 ± 0.61						
N95	30*	1.69 ± 0.38						
	85*	3.45 ± 0.48						
	150	5.64 ± 1.94						

MS2 data for the N95 respirator are taken from Balazy *et al.*, 2006b for 30 and 84 l min^{-1}.

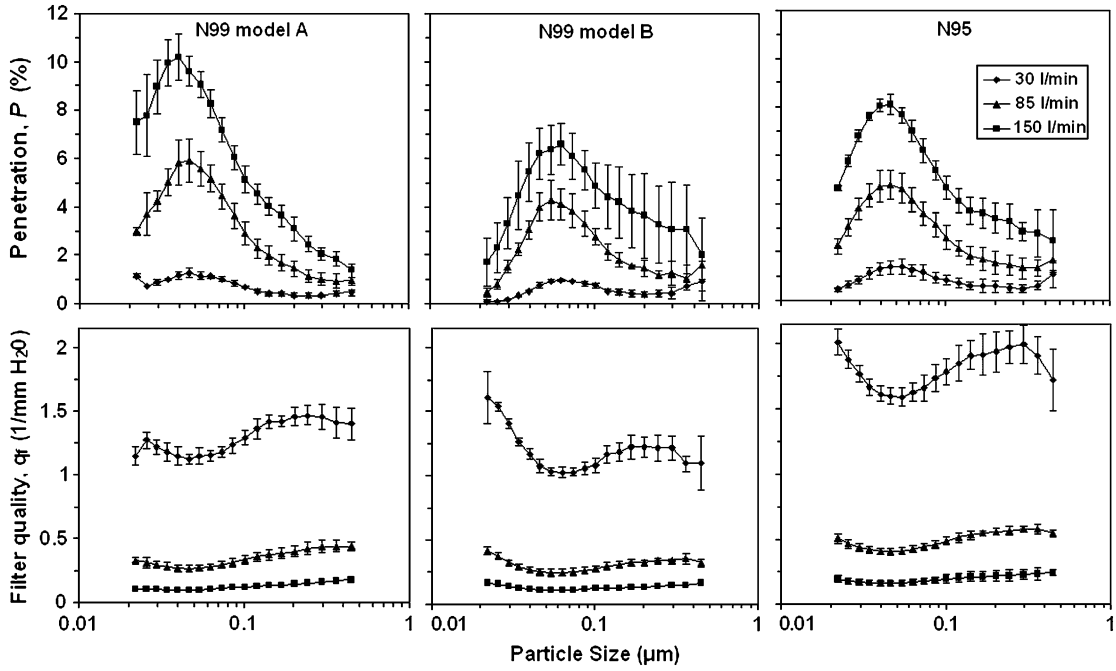

Fig. 3. Aerosol penetration and filter quality factor of three respirators as a function of the particle size and inhalation flow rate for NaCl challenge aerosol.

specifically for particles from 0.1 to 0.5 μm). For N99 Model B, peak penetrations were 6.6, 4.3 and 1.0% at Q = 150, 85 and 30 l min^{-1}, respectively. The mean penetration at 85 l min^{-1} was 2.4% for particles 0.02–0.5 μm and 1.7% for 0.1–0.5 μm. The N95 respirator filter, peak penetrations were 8.1, 4.8 and 1.4% at each respective inhalation flow rate. At Q = 85 l min^{-1}, mean penetrations 2.9% (integrated 0.02–0.5 μm) and 1.7% (integrated >0.1–0.5 μm). Mean penetration was significantly higher for all three respirators when taking into account ultrafine sizes as compared to those >0.1 μm. The N95 data are consistent with previous observations using N95 FFRs (Balazy *et al.*, 2006a). It is apparent from Fig. 3 that penetration was quite similar between respirators even though respirator classes differed (N95 versus N99).

Table 1 shows the pressure drop values across the filter for each respirator and airflow. The N95 FFR demonstrated the lowest resistance at each airflow while N99 Model B possessed the highest. The pressure drop values are consistent with those reported previously for N95 FFRs and N99 filter cartridges by Martin and Moyer (2000). Although Δp differed, particle penetrations appear similar. This can be explained by the charge densities carried by the filter material. Use of electret filters (with charged fibers) allows for increased filter efficiency without increased breathing resistance. The tested N95 filter likely possesses a higher charge density and lower packing density than the N99 respirators.

The size-fractioned filter quality factor (q_f) is also shown in Fig. 3 as a function of the NaCl particle size and inhalation flow rate. It is not as dependent on the particle size as filter penetration. While q_f is similar between respirators operating at 85 and 150 l min^{-1}, the N95 demonstrates higher quality factor at 30 l min^{-1} due to its lower pressure drop: 2.7 ± 0.10 mmH_2O as compared to 3.9 ± 0.20 and 4.5 ± 0.15 mmH_2O measured for N99-A and –B, respectively. The q_f value determined for a specific particle sizes of 0.1 and 0.3 μm were similar to the mean value obtained for the size range of 0.02 to 0.5 μm. Filter quality factor was significantly lower for all particle sizes (integrated mean, 0.02–0.5 μm) than for particles calculated specifically for >0.1 μm.

The utility of filter quality factor in assessing the respirator filter performance is not presently established. One reason is that respirator performance also depends upon face-seal leakage, which is not accounted for in filtration studies. Whether face-seal leakage and filter resistance are related in FFRs has not been thoroughly investigated. Although wearer comfort is expected to increase with increasing q_f for specific filtration efficiency, this has not been quantitatively studied, and physiologically meaningful differences of filter quality factor have not been assessed. Quality factor has been used previously as a tool for comparing respirators. Han (2000) ranked respirator performance using q_f at inhalation flow rates from 10 to 85 l min^{-1} and utilized a plot of flow rate versus q_f to compare FFR. Also,

Chen *et al.* (1992) utilized q_f to compare performance of filtering facepieces and respirator cartridges.

MS2 phage challenge aerosol. Table 1 and Fig. 4 present the mean penetration values for MS2 virus with a designated particle size range of 0.02–0.09 μm. Strongly populated by single virions as well as virus aggregates, this size range accounted for ~82% of the upstream particle count. Airflow had a strong effect: mean penetration at 150 l min^{-1} exceeded that at 30 l min^{-1} by a factor of 5.3 (N99-A), 5.9 (N99-B) and 3.3 (N95). Similar to the trend observed with the NaCl aerosol in this study [and the conclusion made by Balazy *et al.* (2006a,b) for N95 FFR], at Q = 85 l min^{-1}, the MPPS was < 0.1 μm for all three respirators; peak penetrations were 4.3% (N99-A), 4.6% (N99-B) and 4.3% (N95, data from Balazy *et al.*, 2006b), while mean penetrations were 3.4, 3.3 and 3.5%, respectively. Figure 4 demonstrates relatively high variability in the penetration of the N95 respirator at Q = 150 l min^{-1}, but—again—the trend is consistent with previous observations at 30 and 85 l min^{-1} (Balazy *et al.*, 2006b).

Bacillus subtilis and T4 phage challenge aerosols. Table 1 and Fig. 5 present the data for N95 respirator filter challenged with the *B. subtilis* phage and T4 phage viruses. The effect of airflow on penetration is readily apparent with the overall mean penetration at 150 l min^{-1} exceeding that at 30 l min^{-1} by an average factor of 6.6 (*B. subtilis* phage) and 9.5 (T4 phage). At 85 l min^{-1}, peak penetrations were 3.4% for the *B. subtilis* phage aerosol and 2.6% for the T4 phage aerosol occurred at 0.04 μm, which is smaller than the mobility sizes of single virions of *B. subtilis* and T4 phages estimated based on their physical dimensions. This is attributed to the presence of remnant solutes, biological fragments and impurities associated with preparation and freeze drying. Penetration at the single virion mobility diameter, calculated specifically at 0.1 μm and Q = 85 l min^{-1} were 1.9% (*B. subtilis* phage) and 0.95% (T4 phage). Low particle counts for the T4 challenge aerosol resulted in large standard deviations in penetration measurements beyond ~0.12 μm.

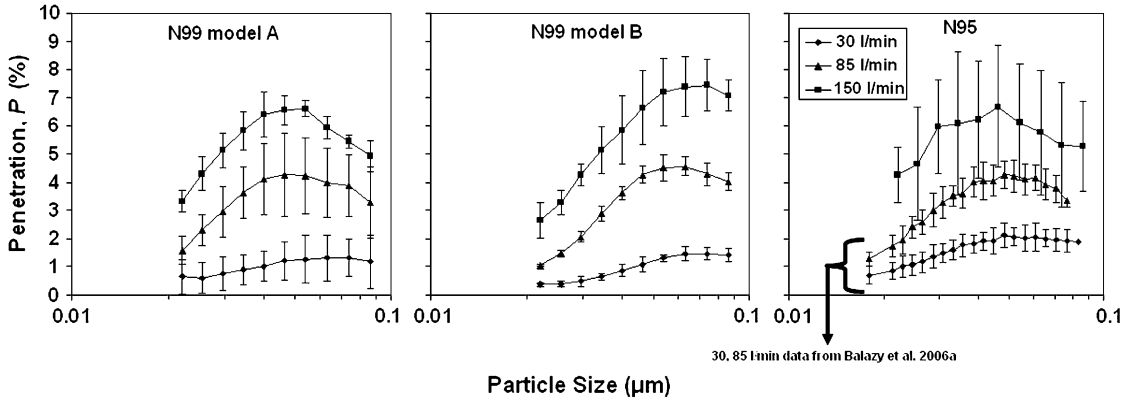

Fig. 4. Aerosol penetration through three respirators as a function of the particle size and inhalation flow rate for MS2 bacteriophage challenge aerosol.

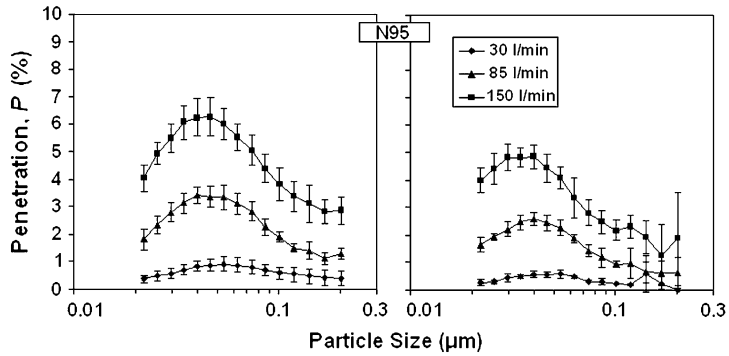

Fig. 5. Aerosol penetration through N95 respirator as a function of the particle size and inhalation flow rate for two challenge viruses: *Bacillus subtilis* bacteriophage (left) and T4 bacteriophage (right).

Between and within-respirator comparisons

The penetration of NaCl aerosol in two particle size ranges was compared between respirators as shown in Fig. 6. Although we expected differences in filtration between respirator classes (N99 was expected to be more efficient in collecting particles than N95), no significant differences in mean penetration were observed for the range of 0.02–0.5 μm (Fig. 6a) or 0.1–0.5 μm (Fig. 6b). However, due to the small sample size, we fall short of concluding that the performance of N99 FFRs is generally no better than that of N95 FFR for the particles up to 0.5 μm. It seems more reasonable to state that filtration of a 'better' N95 FFR may approach the performance of some N99 FFR models over the particle sizes observed here when measured by count.

Mean penetration was also compared by particle size range and differed significantly; when analysis was limited to particles of >0.1 μm, mean penetration for all three respirators was significantly lower ($P = 0.01$) than for particles ranging from 0.02 to 0.5 μm. The greatest contribution to penetration occurred at <0.1 μm for all three respirators. Utilizing a protocol that can also measure the ultrafine component of the test aerosol may result in discovering significantly higher filter penetration (by particle count) than it is anticipated. These observations do not mean that the tested respirators fail to comply with their respective NIOSH certification criteria because the NIOSH certification protocol uses a different method to measure aerosol concentrations to calculate filter penetration (DHHS, 1995).

While no differences were observed between respirators when comparing mean penetration of MS2 aerosol in the designated particle range of 0.02–0.09 μm (see Fig. 7), we also compared the penetration of NaCl to (i) the MS2-containing aerosol for each respirator over the integrated size range of 0.02–0.09 μm (Fig. 8) and (ii) the two larger phages *B. subtilis* and T4 at their estimated mobility diameter of 0.1 μm (see Fig. 9). The Figures show comparisons for $Q = 85\ \mathrm{l\ min^{-1}}$. These served as direct comparisons of inert particle penetration to that of aerosols containing biological particles over the same mobility diameters. Two differences were observed. At $150\ \mathrm{l\ min^{-1}}$, there was a significant difference in penetration between MS2 (5.4%) and NaCl (8.5%) for N99 Model A over the integrated size range of 0.02–0.09 μm ($P = 0.01$, not shown in figure). Also, we found a significant difference between NaCl and T4 phage at $85\ \mathrm{l\ min^{-1}}$ ($P = 0.005$)

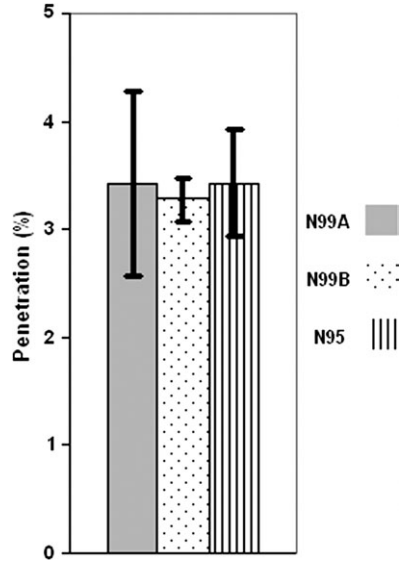

Fig. 7. Between-respirator comparison: mean penetration of MS2 (integrated for the size range of 0.02–0.09 μm) at $85\ \mathrm{l\ min^{-1}}$.

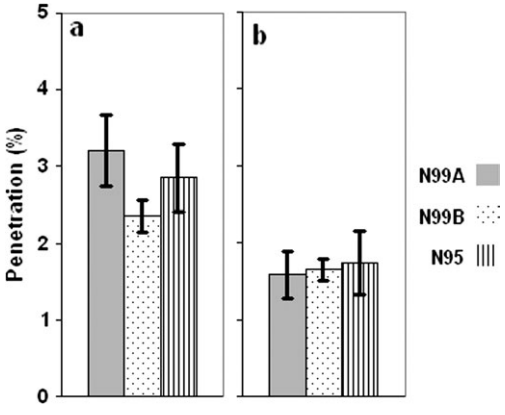

Fig. 6. Between-respirator comparison: mean penetration of NaCl [integrated for the size range of 0.02–0.5 μm (a) and 0.1–0.5 μm (b)] at $85\ \mathrm{l\ min^{-1}}$.

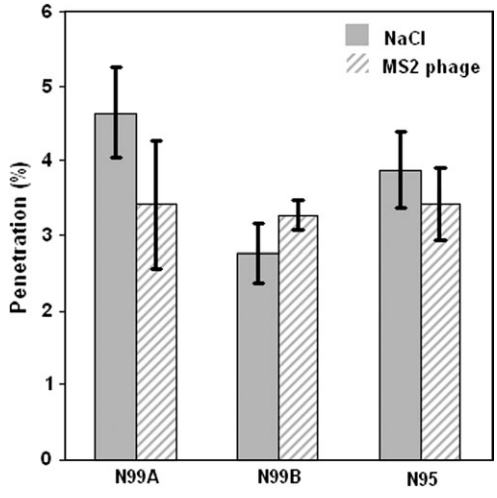

Fig. 8. Within-respirator comparison: mean penetration of NaCl and MS2 (integrated for the size range of 0.02–0.09 μm) at $85\ \mathrm{l\ min^{-1}}$.

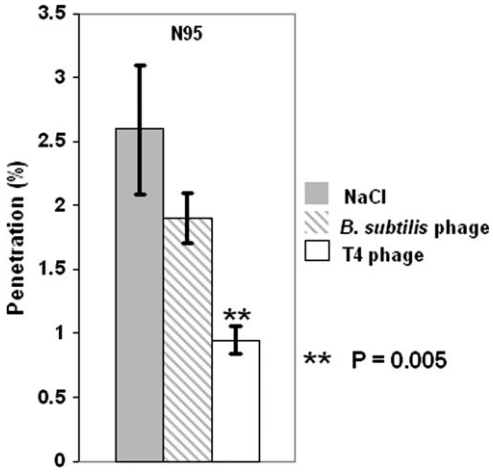

Fig. 9. Within-respirator comparison for N95 at 85 l min^{-1}: mean penetration of NaCl compared to *Bacillus subtilis* phage and T4 phage at 0.1 μm.

where T4 phage penetration was 0.95% compared to 2.6% for NaCl (Fig. 9) for the N95 FFR. Overall, no biological aerosol penetration exceeded that of inert aerosols.

Several properties of airborne virus particles may have influenced filtration in this study and could have contributed to the observed—although inconsistent—differences between the inert and biological aerosols. Particle parameters that effect diffusion, the electrostatic collection mechanism or particle adhesion to the filter fibers are believed to be relevant. Particle shape may affect virus particle filtration since it can influence its polarization and formation of dipole charges in an electrical field (Flagan, 2001). Also, shape can influence particle drag by altering terminal velocity toward an influencing fiber, changing the probability of capture (Flagan, 2001). Dynamic shape factors that aid in describing behavior of airborne virus particles have not been investigated. Lastly, shape may also influence filtration through particle rebound. Boskovic *et al.* (2005, 2007) recently observed differences in filtration efficiency between spheres and perfect cubes of the same electrical mobility diameter up to 0.3 μm. Greater penetration of cubes was ascribed to differences in rebound probability during tumbling at the fiber surface. It is not presently known how the shape of virus aerosol particle may affect its rebound during filtration.

Electrical properties of virions may also influence filtration. With a neutralized aerosol, the virus particle permittivity or dielectric constant is of interest. This represents the ability of a particle to polarize when in an electric field. The degree of polarization will be proportional to the force of attraction between the particle and the influencing fiber (the polarization force). It has been shown theoretically and experimentally that particles with high-dielectric constant are captured by an electret filter with greater efficiency than those with low-dielectric constant (Oh *et al.*, 2002; Yang and Lee, 2005; Wei *et al.*, 2006). The dielectric constant of NaCl is ~6. While the dielectric constant of the tested virions is not known, similar size virions have been estimated to have dielectric constants of >55 (Aristides *et al.*, 2007; Lepizco-Encinas and Rito-Palomares, 2007).

CONCLUSIONS AND FUTURE WORK

The penetration of four challenge aerosols through three N-type FFRs at three inhalation flow rates was determined. Challenges remain in aerosolizing viruses with the intention of creating a monodisperse aerosol consisting of single virions. As seen in this and other studies, remnant solutes, biological fragments and the possibility of aggregate formation can significantly contribute to the resulting particle size distributions.

Inhalation airflow had a significant impact upon particle penetration. The primary mechanisms of ultrafine particle capture—diffusion and electret charge interaction—are heavily influenced by the filter face velocity. Since the selected 150 l min^{-1} flow may underestimate the 95th percentile PIF during occupational tasks, additional study seems feasible in this area to better define a very severe or worst case condition. Also, further study of respirator penetration during cyclic breathing with high PIFs is needed.

The pressure drop across the filters was determined and the filter quality factor calculated providing information on relative performance of the respirator filters. However, the salience of this information without reference to performance during respirator wear is limited. Investigation of whether filter quality factor is predictive of actual workplace protection would determine whether it is a meaningful metric of FFR performance.

The MPPS was <0.1 μm for all aerosol challenges. This has been demonstrated previously for electret-type filter materials using physiologically relevant airflows. As a corollary, we also observed that overall respirator penetration increases significantly—when measured by count—if the ultrafine fraction of the test aerosol is properly detected and included in the integration. This finding is important because the NIOSH filter certification protocol assumes an MPPS of 0.3 μm (by mass) and cannot adequately measure aerosol particles <0.1 μm due to limitations of photometry.

We observed that a better performing N95 FFR can approach the filtration performance of some N99 FFRs over the tested particle size range. However,

this should be considered with caution and not generalized because the presented results were obtained for a single model of N95 compared to two specific models of N99.

Overall, viral penetration through the tested FFRs did not exceed that of inert NaCl aerosol. We observed a difference between inert and bioaerosol filtration where NaCl penetration exceeded that of MS2 (for N99 Model A at 150 l min^{-1}) and that of T4 phage (for N95 FFR at 85 l min^{-1}) which may be attributed to a number of causes. The results suggest that inert aerosols may generally be appropriate for modeling filter penetration of similarly size viruses.

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APPENDIX A3

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Research Article

Differentiating Between Physical and Viable Penetrations When Challenging Respirator Filters with Bioaerosols

The feasibility of a novel testing protocol that allows differentiating between the physical (total) and viable bioaerosol penetrations through respirator filters was investigated. Three respirator models – two conventional N95 filtering-facepiece respirators (FFR) used as controls and one P95 iodinated polymer FFR with antimicrobial properties – were challenged with aerosolized MS2 bacteriophage virus. Physical ($P_{physical}$) and viable (P_{viable}) filter penetrations were simultaneously measured with the FFR sealed on a manikin at a constant inhalation flow rate of 85 L/min. Separate testing was performed on specially-manufactured P95 filter swatches with *(i)* no iodinated resin additive and *(ii)* "high" amount of the additive to determine whether it influenced filtration behavior of the P95 respirator. Bioaerosol collection on the N95 FFR filters fell in the range consistent with previous studies featuring about 2% penetration for MS2 and a peak around ~5%. The P95 iodinated polymer respirator was found to be highly efficient, attributed in part to the iodinated resin powder which in separate swatch tests was found to increase the filter collection efficiency. No statistically significant differences were observed between penetration values obtained for total and culturable viruses for the two control respirators. Similarly, no difference was observed for the iodinated respirator, which suggested that the microbial inactivation effect was of insufficient magnitude to be detected or was not present for viral particles that penetrated the filter. Possible "long-term" inactivation effect of the iodine-based additive on the viable viruses, which were captured on the filter over time, was beyond the scope of this study. The novel testing protocol appears to be an adequate tool for evaluating respirators designed to protect against bioaerosol particles. Further improvement may be considered with respect to the aerosolization method for viable microorganisms.

Keywords: Respirator; Penetration; Bioaerosol; Viable; Physical

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1 Introduction

Bioaerosol exposure may pose numerous hazards in residential, occupational and ambient environments and possesses considerable public health significance. Bioaerosols are known to cause infectious diseases, allergic sensitization, and acute and chronic toxic effects [1]. Occupations with potential bioaerosol exposure may include – but are certainly not limited to – healthcare workers, wastewater and solid waste workers, biomedical researchers, workers in environments employing biomedical technology, farmers, veterinary workers, and food preparation workers [2, 3].

Air-purifying respirators (APRs) are commonly used for reducing workplace exposures in situations where source control is not present or is inadequate. However, governmental guidelines for respirator selection in occupational environments (if they exist) do not generally address bioaerosols or infectious agents. In the United States, guidance for selecting personal protective equipment (PPE), including respirators, is available only for some specific biological agents and environments with bioaerosol exposure, for example, SARS [4], avian influenza [5, 6], and infectious or pathogenic agents in medical and laboratory settings [7]. In addition, recommendations for selection of respiratory protection devices for mold exposure during remediation activities are available [8]. However, no unified strategy for selecting respiratory protection devices against bioaerosols has been developed and adopted in the US or worldwide [9, 10]. Due to the anthrax attacks of 2001, the SARS outbreak of 2003, and the current threat of pandemic influenza, international organizations, governments, and industry are increasingly focused on the development and performance evaluation of disposable and reus-

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Abbreviations: FFR, filtering-facepiece respirators; **NIOSH,** National Institute for Occupational Safety and Health; **CV,** coefficient of variation

able respiratory protection devices that would be efficient against airborne biological particles (mostly viruses and bacteria). The need for billions of disposable respirators for workers and general population in the event of pandemic or terrorist attack has been recently recognized in many countries, which began their stockpiling. Given the tremendous resources involved in the preparedness programs nationally and internationally, it is especially important to determine the efficiency of selected respirators against the bioaerosol agents of concern.

Investigators assessing the collection efficiency of respirator filters have observed that *(i)* inert aerosol particles (non-biological surrogates such as sodium chloride) sufficiently mimicked filtration of bioaerosol particles of similar aerodynamic diameter; and *(ii)* direct reading instruments used for measuring the filter penetration produced similar results when compared to culture-based methods for a given bioaerosol [11 – 16]. These investigations were performed primarily using challenge bioaerosol particles of around 1 μm or larger. Two other studies addressed smaller particles while comparing the respirator filter efficiency of bioaerosol challenges such as MS2 bacteriophages versus inert (non-biological) aerosol particles in the ultrafine fraction (< 0.1 μm) [17, 18]. The viral particle size range addressed in these studies includes the most-penetrating particle size identified for respirators with electrically charged ("electret") filters when challenged with neutral aerosols [19 – 21].

Bioaerosols possess an added layer of complexity when compared to inert particles with respect to respirator selection [9, 22]. Microbial transmission, viability, proliferation, and pathogenicity must all be taken into account. Health effects from bioaerosols may relate to toxic components – which are present whether a microbe is viable or not – such as bacterial cell wall lipopolysaccharides [3]. Alternatively, health effects may relate to infectious potential or toxin production during infection. Also, the infectious dose of a given biological agent spread in aerosol form may be very low, which makes a hazard disproportionate to its airborne mass or count concentration and precludes the use of a traditional exposure limit.

Among the approaches recently introduced for improving the effectiveness of respiratory protection against bioaerosols, one is based on adding biocidal components to the filter medium in order to inactivate viable microorganisms. These respiratory protection devices (known as "antimicrobial respirators") aim at inactivating either microorganisms penetrating through the filter ("instant killing" effect) or those captured by the filter (effect occurs over the time of filtration) [23]. Filter media with additives such as halamine, silver or titanium-based nanomaterials, or iodinated powders have a potential for utilization to increase durability, protection, and aid in filter decontamination [24 – 30]. Adequate methodologies and protocols are needed to evaluate the performance of these newer respirator filters with a specific focus on testing their antimicrobial capability. At present, professionals are debating whether assessing filtration specifically for the viable microorganisms should become an appropriate part of the respirator performance evaluation requirements [23].

To fully assess the total particle filtration efficiency and antimicrobial effect of a bioaerosol filter interaction, an ideal protocol should differentiate between physical (often referred to as "total") filtration efficiency and viable filtration efficiency. Physical filtration efficiency ($\eta_{physical}$) is defined as the percentage of incoming particles/microorganisms that have been collected by the filter, regardless of their viability. Viable filtration efficiency (η_{viable}) is usually derived using culture-based analysis and is the percentage of culturable microorganisms collected by the filter. For a respiratory protective device with no antimicrobial capability, $\eta_{physical}$ and η_{viable} should be the same. For a filter to exhibit biocidal effect, η_{viable} must exceed $\eta_{physical}$. In the latter case, both the physical and viable efficiencies should be known. Figure 1 schematically represents a notional example using an antimicrobial filter challenged with bioaerosol particles some of which are viable. As seen from this example, $\eta_{physical}$ is:

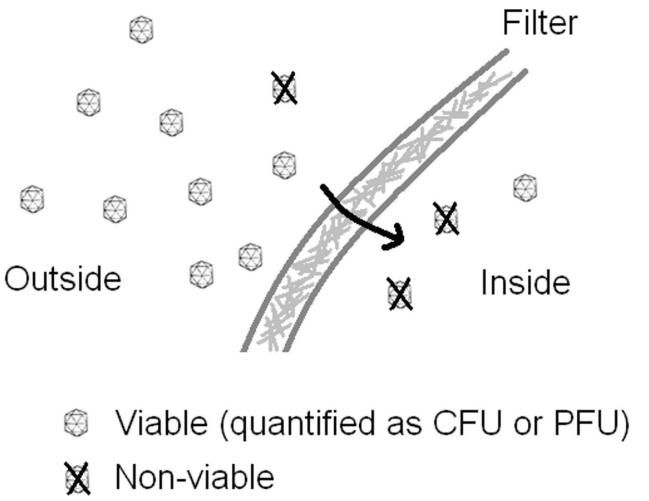

Figure 1. Illustration of physical and viable filtration.

$$\eta_{physical} = \left(1 - \frac{3}{10}\right) \times 100 = 70\% \quad (1)$$

and η_{viable} is:

$$\eta_{viable} = \left(1 - \frac{1}{9}\right) \times 100 = 88\% \quad (2)$$

The 18% difference between the collection efficiencies of total and viable bioaerosols occurring due to the respirator antimicrobial properties translates into a 2.5-fold difference (30 versus 12%) in bioaerosol penetration through the filter media ($P = 100\% - \eta$).

Existing respirator performance testing standards in the US do not fully address $\eta_{physical}$ and η_{viable}. According to the protocol of the Center for Devices and Radiological Health (CDRH) within the US Food and Drug Administration (FDA), surgical masks and respirators classified under 21 CFR 878.4040 [31] and intended for disease prevention are evaluated by undergoing two tests [32]. The first test uses 0.1 μm polystyrene latex (PSL) spheres and serves to measure the total (physical) particulate filtration efficiency [33]. The second test utilizes aerosolized *Staphylococcus aureus* bacteria and aims at measuring the viable filtration efficiency [34]. However, because the challenge aerosol particle sizes in the above two tests differ by an order of magnitude – 0.1 μm compared to ~1 μm – the resulting filtration efficiency measures are not comparable and, even when coupled, are not informative in assessing the performance of respirators with claimed antimicrobial properties. The National Institute for Occupational Safety and Health (NIOSH) certifies the performance of respirator filters based on measuring the total filtration efficiency of two non-biological challenge aerosols: sodium chloride, and dioctylphthalate (DOP). Both challenge aerosols have mass

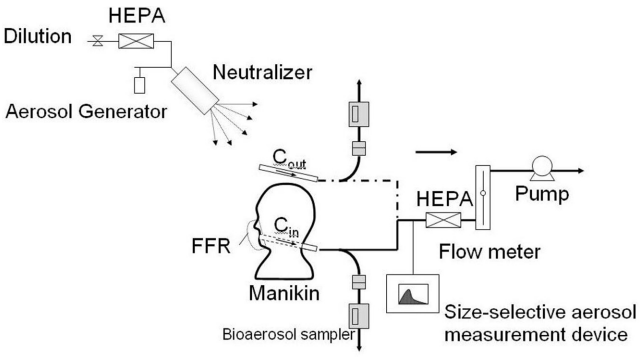

Figure 2. ■Please give legend■

median aerodynamic (MMAD) sizes of ~0.3 μm [35]. The NIOSH certification protocol does not assess viable filtration efficiency.

Thus, no existing respirator test methodology/protocol differentiates between $\eta_{physical}$ and η_{viable}. The purpose of this study was to develop and assess the feasibility of a test protocol that integrates common instruments and approaches and enables the above-mentioned differentiation when respirator filters are challenged with viable bioaerosol particles, including single virions representing the most penetrating particle size for electret filters.

2 Materials and Methods

The test respirators were challenged with viable aerosol of MS2 bacteriophage (ATCC, 15597-B1) obtained from the American Type Culture Collection (ATCC, Rockville, MD), and the physical and viable filter penetrations were determined simultaneously. The experimental facility is schematically shown in Fig. 2. Physical filter penetrations were measured using a manikin-based protocol described in our earlier papers [18 – 20]. The particle penetration rather than collection/filtration efficiency seems to be a convenient measure to compare performance of highly efficient filters (with η close to 100%); therefore, it was used for the data presentation in this paper.

MS2 challenge aerosol was generated with a 6-jet Collison nebulizer (BGI Inc., Waltham, MA) – widely used in bioaerosol studies, e.g., [36 – 44]. Dried with clean air and charge-equilibrated to a Boltzmann charge distribution with a Kr^{85} sealed source (Model 3054, TSI Inc., Minneapolis, MN), the challenge bioaerosol entered a 0.096 m^3 test chamber that housed a manikin with the tested respirator sealed on it. A constant volumetric flow of 85 L/min was drawn for 15 min through the probed manikin. Size-fractioned concentrations of aerosolized MS2 bacteriophage were measured outside ("upstream") and inside ("downstream") of the respirator using a Wide-range Particle Spectrometer (WPS, model 1000 XP, MSP Corp., Shoreview, MN). The lower particle size limit of the WPS is 10 nm. Since its first five measurement channels (10 – 17 nm) recorded too few particles (<20 per channel) inside the respirator and represented sizes considerably below the size of MS2 virions, the data were plotted starting from 17 nm. Extending the particle size scale up to 100 nm enabled us to include the nominal MS2 virion size as well as most-penetrating particle size range known for respirators utilizing electret filter media when challenged with neutral particles [18 – 21]. The aerosol chamber was enclosed in a Biosafety Level II cabinet (SterilchemGARD, Baker Co., Sanford, ME).

Respirator leakage was not assessed as the respirators were sealed to manikin faces and leak-tested. Also, since we studied the initial filtration, the study did not aim at quantifying the change in the filter efficiency over time due to particle loading.

Physical filter penetration was calculated as a ratio of the downstream, C_{down}, and upstream, C_{up}, concentrations and plotted as a function of the particle size:

$$P_{physical} = \frac{C_{down}}{C_{up}} \times 100\% \quad (3)$$

Bacteriophage MS2 was used as the challenge aerosol because of its small particle size – ~28 nm in physical diameter [45, 46] – ease of preparation, low pathogenicity, and history of utilization as a simulant of pathogenic viruses [47, 48]. Methods to prepare the MS2 bacteriophage for aerosolization have been described elsewhere [18, 20]. MS2 aerosol suspensions had a typical phage titer of 10^9 plaque-forming units of MS2 per milliliter of solution (PFU/mL) determined using a modified plaque assay [49].

In parallel to the real-time measurement, MS2 bacteriophages were collected outside and inside the respirator using 25 mm gelatin filters with a 3 μm pore size (Sartorius AG, Göttingen, Germany obtained through SKC, Inc.) within sterilized 25 mm filter holders (SKC, Inc., Eighty Four, PA) at a flow rate of 4 liters per minute, calibrated pre and post-sampling. This method has shown good collection efficiency (>93%) and maintenance of MS2 viability [50 – 52]. Gelatin filters were then dissolved in sterile filtered water and mixed (Touch mixer-Fisher Scientific Inc.). Aliquots of dissolved gelatin filter extract were serially diluted and used for plaque assay to determine the number of airborne culturable MS2 virions (PFU per cm^3 of air sampled) using *Escherichia coli* (ATCC 15597, strain C3000) as the host organism.

The viable filter penetration was determined as the ratio of concentrations of culturable viruses downstream ($C_{V\,down}$) and upstream ($C_{V\,up}$), respectively:

$$P_{viable} = \frac{C_V down}{C_V up} \times 100\% \quad (4)$$

Based on the WPS measurements, the electrical mobility diameter of 22 to 29 nm was designated for MS2 virions. This particle size range was selected to represent single MS2 bacteriophage virions, which have been observed to have an electrical mobility diameter of approximately 24 nm and because it matched discrete channel particle size boundaries used by the WPS [53]. The mean penetration for particle sizes integrated from 22 to 29 nm in electrical mobility diameter was calculated to obtain the physical penetration in the size range of viral particles ($P_{physical\text{-}virus}$).

Two commercially available conventional N95 filtering-facepiece respirators (FFR), produced by different manufacturers, and one commercially available iodinated polymer P95 FFR utilizing a filter treated with iodinated resin powder (10 g/m^2) were tested with respect to their filtration of viruses – total and viable. The mean and standard deviation for P_{viable} was calculated for each respirator and compared to the $P_{physical\text{-}virus}$ using t-test (if normal) or the Mann-Whitney test (if non-normal). The conventional respirators served as controls to validate the test method and were expected to demonstrate that P_{viable} and $P_{physical\text{-}virus}$ did not differ significantly.

Additionally, swatch tests were performed primarily to investigate if the presence of the antimicrobial additive influenced the media's physical filtration characteristics. Two filter swatches were specially manufactured utilizing the tested P95 filter material: one

with no iodinated resin added and one with a 3.5-fold greater amount of the powder per unit surface area (35 g/m^2) as compared to the commercially available FFR. These filter swatches (as well as the swatch of the commercial P95 with a filter material that had undergone "normal" treatment) were tested following a modified protocol, in which they were mounted inside 47 mm stainless steel filter holders (model 2220, Gelman Life Sciences, Ann Arbor, MI) – as an alternative of sealing them on the manikin face – and challenged with a NaCl aerosol.

Five tests were performed for each commercial respirator model (two control N95's and one P95) as well as for specially manufactured filter swatches. The respirator tests used a constant airflow of 85 L/min – the same used by NIOSH in their certification protocol. The swatch tests were conducted at a much lower flow rate (~1.1 L/min) to achieve the same face velocity as that in the respirator test (~7 cm/s).

When any additive is incorporated in the respirator filter material, especially of potential biocidal properties, it is important to identify whether this additive may be released (as a gas or aerosol) during breathing and inhaled by a wearer. To quantify the iodine release from the iodine-treated respirator filter, a separate experiment was designed. A swatch of filter material with the higher amount of iodine powder (35 g/m^2) was exposed to a constant air flow and the overall iodine release (in mg) was measured as a function of time. The test system consisted of a vacuum pump and timer as well as the necessary tubing and flow meters capable of measuring the required flows. A flow rate of 42.5 L/min was established through a 100 cm^2 filter area over an 8-h period. The iodide released was measured using an HPLC (model DX600, Dionex Corporation, CA). The main components of this instrument were an auto sampler, chromatography oven, pulsed electrochemical detector containing a silver working electrode and silver/silver chloride reference electrode, gradient pump, and compressed helium gas tank with regulator. The quantity of iodine was initially measured in parts per million and then transformed into an iodine concentration (mg/m^3). The measured cumulative mass of the iodine downstream of the filter represented a conservative scenario aiming at simulating *(i)* a moderately to hard work breathing during a full work shift and *(ii)* excessively powerful source of iodine that can potentially be released. The instrument's limit of detection was 1.6610^{-3} mg/m^3. The measurements were conducted in six replicates.

3 Results

In Fig. 3, the size-fractioned physical penetration of the challenge aerosol (measured from 17 to 100 nm) is shown superimposed by the viable penetration in the virus-designated mobility-based diameter of 22 to 29 nm. Each area represents the mean penetration plus and minus one standard deviation. As expected, no statistically significant differences between P_{viable} and $P_{physical\text{-}virus}$ were observed for the two control respirators calculated as integrated means over the particle sizes from 22 to 29 nm ($p > 0.05$). Additionally, no significant difference was observed between P_{viable} and $P_{physical\text{-}virus}$ for the iodinated P95 respirator. The first control N95 respirator demonstrated a $P_{physical\text{-}virus}$ of 1.5 ± 0.26% (with a coefficient of variation, CV, of 0.17) and a P_{viable} of 1.8 ± 0.83% (CV = 0.46) (see Fig. 3a). Physical penetration for the second control N95 respirator, shown in Fig. 3b, was 1.82 ± 0.37% (CV = 0.20) and viable penetration was 1.7 ± 0.78% (CV = 0.46). As seen from Figure 3c, the iodinated polymer respirator demonstrated very efficient overall filtration with a physical penetration of 0.012 ± 0.006% (CV = 0.5) and viable penetration of 0.016 ± 0.001% (CV = 0.06).

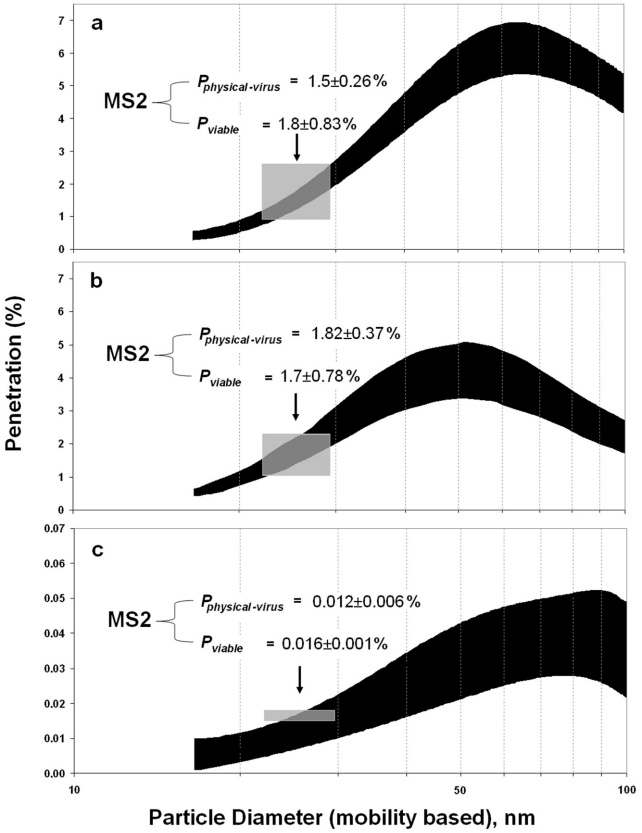

Figure 3. Size-fractioned $P_{physical}$ (in black) superimposed by P_{viable} (in grey). $P_{physical\text{-}virus}$ was determined specifically for the virus-designated mobility-based diameter of 22–29 nm. The plots are bounded by the mean penetration ± 1 SD. (a) Conventional N95 respirator #1; (b) conventional N95 respirator #2; (c) iodinated polymer respirator P95.

Because initial testing of the P95 respirator demonstrated higher filtration than expected for a class 95 FFR, additional filter swatch testing was performed to investigate if the iodinated powder influenced filter behavior. The test results obtained with the iodinated polymer P95 filter swatches of different powder loads challenged with NaCl aerosol are shown in Fig. 4 as best-fit polynomial regressions of mean penetration. The size-fractioned penetration curves are presented respectively for the filter swatches with *(i)* no iodinated resin powder treatment, *(ii)* normal iodine resin powder load of 10 g/m^2 used in the commercially available unit, and *(iii)* high load of 35 g/m^2. The mean penetration integrated for the MS2 particle size range (22 – 29 nm) as well as for the entire size range of interest (17 – 100 nm) differed significantly ($p < 0.01$) between the untreated and treated filter swatches ($P_{untreated} > P_{treated}$).

The iodine release by the constant inhalation air flow, detected downstream of the filter treated with 35 g/m^2, produced a time-weighted average (TWA) air concentration of 0.04 mg/m^3. This is approximately 4% of the applicable occupational exposure limit for iodine as defined by the American Conference of Governmental Industrial Hygienists (ACGIH) and Occupational Safety and Health Administration (OSHA) [54, 55]. Iodine release ranged from ~3 μg/m^3 in the first hour and peaked at a plateau of ~0.1 mg/m^3 at between 6 and 8 h of testing.

4 Discussion

The observed size-fractioned physical penetration of challenge particles through the N95 respirator filters was consistent with previous studies [17 – 20, 56, 57]. The filtration efficiency of the P95 respirator was greater than expected for a class 95 filter, providing filtration nearing that of a class "100" respirator filter or HEPA filter, which are limited to a penetration of 0.03% by mass of a challenge aerosol [35]. It is important to emphasize that the purpose of this study was not to compare penetration between the selected respirators, but to evaluate and compare $P_{\text{physical-virus}}$ and P_{viable} for a given respirator. To properly place the P95 filtration efficiency in context, it must be compared to other P95 respirators under similar test conditions. We performed an inspection of a cross-section of the P95 filter and observed that the number of filter layers and total thickness were similar to class 99 and 100 respirator filters we have tested in our laboratory. The physical properties of the filter were consistent with its high filtration efficiency.

We hypothesized that the difference in filtration efficiencies can be, at least partially, attributed to the iodine resin powder, which was intended to enhance microbial filtration efficiency. The comparison of the NaCl filtration efficiencies of powder-treated and untreated P95 filter swatches enabled us to test this hypothesis. Figure 4 shows a trend of decreasing particle penetration with increasing loading of iodinated resin powder in the filter media. Particle penetration differed significantly between the untreated and treated filter swatches. We hypothesize that the addition of the powder increased the tortuous path length of particles as they passed through the filter medium and possibly enhanced the electrostatic interaction, thus increasing particle removal associated with the diffusion and polarization mechanisms.

The difference between the viral particle filtration efficiencies determined for the total and culturable counts was not statistically significant. This finding was anticipated for the control N95 FFRs, confirmed by the protocol, and served as a validation for the proposed test method. At the same time, we failed to observe $P_{\text{viable}} < P_{\text{physical-virus}}$ for the iodinated polymer respirator suggesting that a microbial inactivation effect was of insufficient magnitude to be detected or was not present for viral particles that penetrated the filter. We acknowledge that the iodine-based additive may cause an inactivation of viable viruses captured on the filter over time. However, such a "long-term" effect was beyond the scope of this study.

There were notable differences in the variability of P_{viable} among the N95 respirators compared to that among the iodinated polymer P95 respirator (~7-fold in the coefficient of variation). This was likely due to the much higher upstream MS2 bioaerosol concentration and longer sample time used for the P95 FFR. To achieve a sufficiently low limit of detection for P_{viable}, two Collison nebulizers were used simultaneously to generate MS2 aerosol when testing the P95 FFR. This likely resulted in much greater precision for P_{viable}.

As indicated above, the time-weighted average (TWA) concentration of the iodine released from the P95 filters having 35 g/m^2 of iodine was considerably lower than applicable occupational exposure limits. The TWA concentration over eight hours of testing was 4% of the OSHA PEL and ACGIH TLV for iodine [54, 55]. The maximum iodine concentration measured occurred between 6 and 8 h of testing and was ~0.1 mg/m^3. Testing for time intervals longer than 8 h would be appropriate, although it is acknowledged this test assessed a filter with 3.5 times the iodine loading of the commercially available P95 respirator. Because widely differing approaches are being used to impart antimicrobial properties in respirator filter media, protocols to assess the user safety may differ from one product to another. This may be a consideration in future respirator certification or consensus standard requirements.

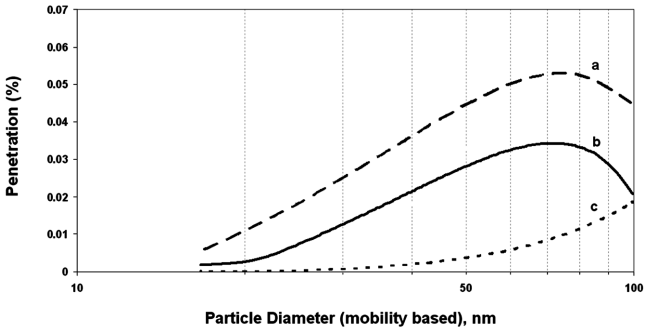

Figure 4. Size-fractioned physical penetration of NaCl for the (a) P95 filter swatch with no iodinated resin; (b) commercially-available P95 respirator filter (10 g/m^2); and (c) P95 filter swatch highly treated with iodinated resin (35 g/m^2). Lines are best-fit polynomial regressions of the data with R^2 values of 0.70, 0.97, and 0.98, respectively.

The proposed protocol appears feasible as a method to assess and potentially differentiate P_{physical} and P_{viable}. There are several advantages to this protocol. Collison nebulizer – the selected method of microbe aerosolization – is inexpensive, has been used in numerous bioaerosol studies, and is capable of producing an aerosol with an ultrafine particle size fraction. Processing of the gelatin filter media for culturing MS2 virions is less labor-intensive than other filter media which may require extensive ultrasonication. Also, measurements for P_{physical} and P_{viable} take place simultaneously, which is a superior study design to sequential measurements.

At the same time, the selection of the Collison nebulizer leads to some limitations of the testing protocol. First, it has been reported that the Collison nebulizer produces a time varying particle size distribution [58], although we did not observe this during the relatively short sample periods used in this study. Second, this aerosolization method may produce MS2 aggregates that can bias the viable filtration measurement [59]. Previous work from our lab [20] suggests that MS2 aggregates do not considerably contribute to the aerosolized viral particles using the proposed methodology. However, work by Hogan et al. [58, 59] suggests the presence of aggregate MS2 clusters may contribute to the total viable particle count. In one study, however [58], a different aerosolization method was used and in the other study [59] the MS2 phage titer was at least 100 times higher than that used in this investigation. Third, contaminants from MS2 bioaerosol preparation and residual solutes aerosolized by a nebulizer may comprise a significant portion of the dried aerosol count thus masking single virions. Therefore, calculations made of P_{physical} may have been influenced by particles other than MS2 virions. To examine whether this occurred, additional study may be required. A promising aerosolization method has recently been tested with MS2 bacteriophages to generate an "ultraclean" aerosol comprised solely of virions [53]. Using electrospray ionization, researchers were able to aerosolize single MS2 bacteriophage virions and accurately measure their electrical mobility diameter while maintaining viability. On the other hand, we anticipate some challenges in implementing the electrospray approach, e.g., preparation of MS2 bacteriophage suspension requires additional purification and concentration steps to obtain a high-titer virus sample.

Besides, equipment cost is approximately ten times that of a Collison nebulizer. Overall, improvement of the aerosolization methodology can considerably enhance the filter testing protocol and provide a more definitive assessment of filtration for a bioaerosol in the ultrafine particle range.

The importance of the upstream bioaerosol concentration as a parameter that influences the utility of the proposed protocol was demonstrated. Too few bioaerosol challenge particles will result in less precise measurements for $C_{v\,down}$, and larger variance in P_{viable} values. Imprecision in either penetration measurement may mask or limit the ability of the protocol to detect differences in $P_{physical\text{-}virus}$ and P_{viable}. This is particularly relevant if testing a highly efficient filter [60]. Considering the low dose required for infection of certain biological agents, the ability to distinguish even small differences between $P_{physical\text{-}virus}$ and P_{viable} is desirable. Protocol parameters that would influence the ability to discriminate $P_{physical\text{-}virus}$ and P_{viable} include the upstream biological particle count, sampling time, number of respirators tested, and the degree to which bioaerosols are masked by contaminants and dried solutes in a nebulizer-generated aerosol. This last factor is probably the primary limitation of aerosolizing bioaerosols with the Collison nebulizer for the purpose of filter testing.

5 Conclusions

The present study investigated the feasibility of a respirator filter testing protocol that enables differentiating between the physical and viable filtration when challenging respirator filters with bioaerosols. We evaluated three respirator models (two conventional N95 FFRs used as controls and one specially treated iodinated polymer P95 FFR) with aerosolized MS2 bacteriophage, and no statistically significant difference was found between $P_{physical}$ and P_{viable} for any model. The treated P95 filter efficiency was greater than expected for a class 95 respirator which was in part attributed to the iodinated resin powder, which apparently improves the filter collection, by enhancing diffusional and electrostatic polarization effects. The physical properties of the P95 filter were more consistent with a class 99 or 100 filter in terms of thickness and number of filter layers. The release of iodine vapor powder from the iodinated polymer respirator filter during inhalation appeared to be well below applicable occupational exposure limits (such as US OSHA's applicable OEL of 1.036 mg/m^3).

The protocol presented in this paper provides a tool for evaluating respirators designed to protect against bioaerosols, both viable and non-viable. At the same time, further modification of the aerosolization system may be warranted because the aerosol nebulized from a viral suspension contains a poorly defined fraction of single viruses and is characterized by a rather broad particle size distribution. This deficiency may affect precision of the filtration measurements. Electrospray ionization shows promise as an alternative means to aerosolize viruses and create a well-characterized, monodisperse challenge bioaerosol suitable for filter testing.

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Research Article: The feasibility of a novel testing protocol that allows differentiating between the physical (total) and viable bioaerosol penetrations through respirator filters was investigated. Three respirator models – two conventional N95 filtering-facepiece respirators (FFR) used as controls and one P95 iodinated polymer FFR with antimicrobial properties – were challenged with aerosolized MS2 bacteriophage virus.

Differentiating Between Physical and Viable Penetrations When Challenging Respirator Filters with Bioaerosols

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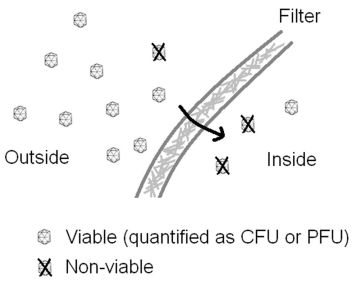
Clean **2008**, *36* (7), 00 – 00



APPENDIX A4

Eninger RM, Hogan, Jr CJ, Biswas P, Adhikari A, Reponen T, Grinshpun SA (2008). Electrospray Versus Nebulization for Aerosolization and Filter Testing with Virus Particles, DRAFT, submitted to *Aerosol Sci Tech*.

ELECTROSPRAY VERSUS NEBULIZATION FOR AEROSOLIZATION AND FILTER TESTING WITH VIRUS PARTICLES

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Keywords: bioaerosol, electrospray, nebulizer, virus, filtration

ABSTRACT

Aerosolization of bacteriophage MS2 virions by nebulization and charge-reduced electrospray were compared during testing of three filter media. Sample swatches were taken from a surgical mask, N95 filtering-facepiece respirator (FFR), and N100 FFR for use in repeated, short-duration (15 minute) penetration tests with bacteriophage MS2 aerosolized by nebulizer and electrospray. Evaluated were (1) the virus suspension preparation protocol, (2) resulting particle size distribution, count stability, and count variability, and (3) the ability to generate culturable MS2 virions. While preparation of the electrospray bacteriophage suspension required additional purification and concentration steps and took more time than the nebulization protocol, it resulted in a much higher titer suspension. The nebulizer produced a polydisperse aerosol; conversely, the electrospray produced a relatively monodisperse aerosol with a count peak at the mobility size of the single virion. The nebulized aerosol particle count was 2.8 times as variable as the electrosprayed aerosol particle count although neither aerosolization method maintained a constant count over repeated 15-minute filter tests. No differences in filter penetration were observed between nebulized and electrosprayed MS2 aerosol particles. Electrosprayed dextrose particles, used as an inert aerosol particle comparator, exhibited higher penetration than MS2 particles in two of the three filter samples, which can be attributed, at least partially, to the difference in dielectric properties of dextrose and virus particles. Both aerosolization methods generated culturable MS2 virions with the electrospray producing an airborne concentration ~20-fold higher than the nebulizer. In general, the electrospray produced cleaner, more stable, and more viable baceteriophage aerosol particles compared to conventional nebulization. The findings of this study are expected to assist

researchers in selecting appropriate generation methods when using viable virus- based challenge aerosol particles.

INTRODUCTION

Ensuring that respirator filters efficiently collect biological aerosol particles is of the utmost importance, and precise methods for filter testing with biological aerosol particles need to be developed (Rengasamy et al. 2004). Nebulization is a common methodology used for aerosolizing biological particles, such as bacteria (Jensen et al. 1992; Grinshpun et al. 1997; Foarde et al. 1999; Johnson et al. 1999; Griffiths et al. 2001; Li and Lin 2001; Mainelis et al. 2005), fungi and fungal fragments (Lin and Li 2003), and viruses (Agranovski et al. 2005, Balazy et al. 2006a, Kim et al. 2007) as it is a relatively inexpensive aerosolization technique and does not require extensive user training or trial and error for correct and reliable operation. Likewise, nebulization is a common aerosolization method utilized for filter testing, e.g., for aerosolizing bacteriophage MS2 (a non-enveloped icosahedral virus) to compare ultrafine inert and biological particle penetration through respirator filters (Balazy et al. 2006a, Eninger et al. 2008a, Eninger et al. 2008b). However, several drawbacks of nebulization for generating ultrafine virus particles have been identified. Nebulization typically generates contaminant particles in the ultrafine size range from dried solutes and biological fragments in the nebulizer suspension. While this contamination is not of great significance when studying micrometer sized biological aerosol particles, ultrafine contaminants may mask the size distribution of virus particles which are of a comparable size (Hogan et al. 2004, 2005). Virus preparation and nebulization may also produce aggregate particles that may bias culture-based filtration measurements because filtration efficiency is a function of particle size. Aggregation of virus

particles prior to and during nebulization is highly dependent on the virus suspension preparation procedure; thus, the size distribution function and viable versus total counts of nebulized virus particles vary between different studies [e.g., Hogan et al. (2005) versus Balazy et al. (2006a)]. In addition, nebulization of virus particles has been observed to produce a time-varying particle size distribution function (Hogan et al. 2005), which is not desirable for filter testing. Most of the nebulized solution flows back to the nebulizer reservoir, but depending upon the chosen solvent, a fraction will evaporate over time causing the solution to become more concentrated (Chen and John, 2001) and changing the aerosolized particle size distribution function.

While nebulization may be non-ideal for the aerosolization of virus particles for filter testing, charge-reduced electrospray (Scalf et al. 2000, Bacher et al. 2001, Hogan et al. 2006) is a promising aerosol generation method that appears to avoid some of the shortcomings of nebulization. With an appropriately prepared virus suspension, charge-reduced electrospray can produce a relatively narrow aerosol size distribution with a clear peak at the nominal virion diameter, without aggregates, appreciable dried solutes and other contaminants (Thomas et al. 2004). The total number concentration of aerosolized virus particles with charge-reduced electrospray has also been shown to be directly proportional to the virus titer in the electrospray suspension (Wick & McCubbin 1999, Hogan et al. 2006).

In this study, we examined the practical aspects of aerosolizing MS2 bacteriophage via nebulization and charge-reduced electrospray for filter testing applications. Each aerosolization method was evaluated and compared with respect to: (1) virus suspension preparation protocol, (2) resulting aerosol concentration, particle size distribution and stability over short time periods (15 minutes), (3) viability, and (4) filtration behavior. The use of inert surrogates in lieu of virus particles for filter media testing was also examined and is discussed in this paper.

MATERIALS & METHODS

Preparation of MS2 virus suspensions for nebulization (Balazy et al. 2006a) and electrospray (Hogan et al. 2006) are briefly summarized below. The nebulizer suspensions were prepared by adding 9 mL Luria-Bertani broth with ultrafiltered water (ASTM reagent water purity type I; ASTM, 2006) to a freeze-dried bacteriophage MS2 vial (ATCC 15597-B1) obtained from the American Type Culture Collection (ATCC, Rockville, MD) and serially diluted to a typical titer of titer of 10^9 plaque-forming units of MS2 per milliliter of solution (PFU/mL) determined with a modified plaque assay (ISO 2000). The electrospray suspension utilized bacteriophage MS2 propagated in bacterial host *Escherichia coli* (ATCC 15597) in a glucose and thiamine minimal media to a titer of 10^{10} PFU/mL. The suspension was then centrifuged (30 minutes at 9000 rpm) and filtered (0.22 µm pore membrane, Fisher Scientific, Pittsburgh, PA). Prior to electrospray aerosolization, 250 mL of the phage suspension was ultracentrifuged (RCF_{max} = 193000g for 3 hours, washed, then centrifuged again for 3 hours) and the virus pellet was resuspended in 1 mL of 10 mM aqueous ammonium acetate (Sigma-Aldrich, St. Louis, MO).

Virion test aerosol particles were generated using a 6-jet Collison nebulizer (BGI, Inc., Waltham, MA) and an electrospray aerosol generator (model 3480, TSI, Inc., Minneapolis, MN). The nebulized droplets were diluted, dried with clean air and charge-equilibrated using a Kr^{85} sealed source (model 3054, TSI Inc.); electrosprayed droplets were dried and charge-equilibrated using a Po^{210} sealed source (model 348002, TSI Inc.). The particle size distribution and concentration were measured using the differential mobility analyzer in a Wide-range Particle Spectrometer (WPS, model 1000 XP, MSP Corp., Shoreview, MN).

Short (15-minute) testing was performed by challenging the filter media with nebulized and electrosprayed MS2 aerosol particles (Figure 1). In these experiments, we used a swatch from the innermost filter layer of three commercially available respiratory protection devices: a surgical mask, an N95 filtering-facepiece respirator (FFR), and an N100 FFR. Five replicate tests of each filter sample were performed.

Nebulizer filter test protocol

MS2 suspensions were aerosolized, diluted, and dried, then charge-equilibrated and passed to a 0.096 m^3 mixing chamber (Figure 1a). The WPS alternated sampling between two identical 47-mm stainless steel filter holders (model 2220, Gelman Life Sciences, Ann Arbor, MI): one with filter media (the "downstream" sample) and one without (the "upstream" sample). Eight samples were drawn at 1 L/min during a 15-min experiment—four each upstream and downstream of the filter. The filter face velocity was 1.7 cm/sec. Based upon the observed mobility diameter of a single MS2 virion (Wick & McCubbin 1999, Hogan et al. 2006), cumulative particle counts were recorded for mobility diameters from 23 to 26 nm. Bias between upstream and downstream sample inlets was checked for each tested filter replicate. The mean upstream and downstream particle counts, corrected for any bias, were then used to calculate filter penetration (P, in %):

$$P = \frac{C_{down}}{C_{up}} \times 100\% \qquad [1]$$

Electrospray filter test protocol

The MS2 suspension was electrosprayed, dried, and charge-neutralized using an internally-mounted Po^{210} source (Figure 1b). Due to the relatively low flowrate of the

electrospray aerosol generator (≤ 2 L/min compared to > 97 L/min for the nebulizer protocol, including dilution flow), the aerosol flow was split and passed directly to the matched filter holders. Unlike the nebulizer protocol, a dilution and mixing chamber was not required because the electrospray dilution flow was only 1 – 2 L/min and the initial droplet size much smaller than that of the nebulizer. The remaining steps matched those used for the nebulizer protocol. We also performed one round of filter testing with electrosprayed dextrose particles (using a dilute dextrose solution in 10mM aqueous ammonium acetate) (Chen et al. 1995) of the same mobility diameter as MS2 bacteriophage.

The characteristics of the test aerosol particles were measured before and during each filter test. This included the particle size distribution, particle count at the nominal virion diameter, and the stability of the particle count over short durations (15 minutes). The stability of the particle count was evaluated in terms of (1) the variability in particle concentration and (2) any trends in particle count over repeated 15-minute filter tests.

MS2 viability test protocol

Each generation method was evaluated for its ability to aerosolize viable bacteriophage MS2 virions with the stock suspensions from the filter tests using a validated method (Jaschhof 1992; Burton et al. 2007; Grinshpun et al. 2007). Aerosolized MS2 bacteriophages were collected using 3 µm pore size 25 mm gelatin filters (Sartorius AG, Göttingen, Germany, obtained through SKC, Inc., Eighty Four, PA) inside sterilized 25 mm filter holders (SKC, Inc.) at a flow rate of 4 L/min for 15 minutes. Gelatin filters were dissolved in sterile filtered water and then mixed (Touch mixer, Fisher Scientific Inc.). Aliquots of gelatin filter extract were diluted and used for plaque assay to determine the number of airborne culturable MS2 virions

(PFU per cm^3 of air sampled) using host *Escherichia coli* (ATCC 15597, strain C3000). The electrospray was tested with two sheath air supplies: dry, filtered air and CO_2, as CO_2 prevents corona formation and ozone generation (Suriyawong et al. 2007) at the electrospray capillary tip.

RESULTS AND DISCUSSION

Virus propagation procedures

The virus preparation protocols were similar for both aerosol generation methods, with several additional concentration and purification steps applied to obtain a high-titer virus sample in aqueous ammonium acetate for electrospray aerosolization. A notable difference in the two protocols was the use of ATCC-supplied freeze-dried bacteriophage for the nebulized aerosol compared to propagating "in-house" the bacteriophage for electrospray suspension. Propagation resulted in a greater volume (liters) and higher titer (~ 10^{10} PFU/mL) than did direct use of freeze-dried bacteriophage MS2. Propagating a high-volume, high-titer stock, however, takes multiple days, as compared to using freeze-dried stock, which is a single day preparation process. Several studies have used both in-house propagated and resuspended freeze-dried MS2 stocks interchangeably for nebulization with no notable differences in virus viability (Balazy et al. 2006a, Eninger et al. 2008b). Conversely, a multi-day propagation process with subsequent centrifugation and ultracentrifugation is critical for preparing high-titer virus suspensions with fewer contaminants for electrospray aerosolization (Hogan et al. 2006).

Aerosol particle size distribution functions

Representative MS2 aerosol particle size distribution functions for each generation method are shown up to 100 nm in Figure 2. The nebulized MS2 aerosol was pronouncedly

polydisperse. With a mode diameter of 49 nm, a geometric mean diameter (d_g) of 60 nm, and a geometric standard deviation (σ_g) of > 1.9, it extended to beyond 400 nm. The shape of the particle size distribution was similar to that observed in previous studies, although it peaked at a higher particle size as compared to Balazy et al. (2006a) experiments, in which MS2 aerosol showed an approximately 30 nm mode. The variability in nebulized MS2 aerosol particle mode diameter is not unusual, as the solute and contaminant content undoubtedly varies from one suspension preparation to another. Often, the solute and contaminant content is sufficiently high such that the presence or absence of viruses in the nebulizer suspension has little influence on the aerosolized particle size distribution (Hogan et al. 2005). Therefore, direct enumeration of virions in the ultrafine size range, such as bacteriophage MS2, from the particle size distribution measured after aerosolizing the virus suspension by nebulization, may lead to spurious results. Combined size selection-virus assay methods have been developed to determine the viable virus particle size distribution function (Hogan et al. 2005); however, these methods do not allow for rapid online virus particle measurement.

The electrosprayed MS2 aerosol particle size distribution function possessed a d_g of 25 nm and σ_g of 1.3. It is shown in Figure 2a up to 100 nm and in greater resolution from 22 to 28 nm in Figure 2b. A clear MS2 virion peak was observed at 24.7 nm, which is in reasonable agreement with the virion peak reported previously for electrosprayed bacteriophage MS2 (Wick & McCubbin 1999, Hogan et al. 2006). The slightly higher geometric standard deviation observed here – as compared to previous studies – is attributable to the use of a low resolution size classifier in place of a differential mobility analyzer (Hogan et al. 2006). The electrospray method produced a narrower size distribution of virion aerosol particles with less interference from dried solutes than did the nebulizer. This is primarily due to the ability of electrospray to

generate initial droplets ~100-200 nm in diameter, which are relatively free of non-volatile solutes and impurities (Kaufman 1998). For such droplets, the average number of virions per droplet was much less than unity, thus the probability of forming dimer and n-mer virion particles was low (Hogan and Biswas 2008a, 2008b). Droplets in which virions were absent contained little to no non-volatile impurities (ammonium acetate readily evaporates); upon evaporation they did not leave residue nanoparticles.

Particle count variability

The upstream aerosol particle count data obtained at four time intervals over the 15-minute test period for each generation method are presented in Figure 3. The phage titer, solute concentration, and impurity content varied slightly between different MS2 suspensions, which affected the concentration and size distribution function of aerosolized virions and impurity residue particles. To account for this, the upstream particle concentration values were normalized for the mean particle counts of each test run. Neither aerosol generation method maintained a constant particle count. The variability of the relative particle concentration of the nebulized aerosol was ~2.8 times greater than that of electrosprayed aerosol particle concentration. A linear regression was performed on the data and is presented in Figure 3 with 95% confidence intervals for the mean and predicted particle counts. Mean particle concentrations of the nebulized aerosol appeared stable (though more variable) over time, whereas the electrosprayed particle count possessed a slight but statistically significant negative slope ($p = 0.01$) of ~ 0.16 % per minute of operation. This implies that although electrospray aerosol generation is much less variable than nebulization, it may not be as feasible for use in long-term virus aerosol studies, e.g. in calibration of long-term virus aerosol samplers (Agranovski et al. 2005).

Filter penetration

No statistically significant differences in particle penetration through filter swatches were observed between the tested aerosol particle generation methods (Figure 4). Penetration was 8.3% and 8.0% through the surgical mask, and 0.72% and 0.70% through the N95 FFR filter sample, for the electrosprayed aerosol particles and nebulized aerosol particles, respectively. Penetration through the N100 filter sample was 0.61% when testing with both aerosolization techniques. Although the nebulized particles in the 23-26 nm range were composed primarily of contaminant and solute residues, their penetration through three tested filter types was remarkably similar to the penetration of clean, non-agglomerated virion aerosol particles of the same size.

Conversely, dextrose aerosol particle penetration through filter swatches was similar to the penetration of electrosprayed MS2 virions for the N100 filter sample, but much greater for both the surgical mask sample (12.2% compared to 8.3%) and the N95 filter sample (1.2% versus 0.73%) – a difference of 1.5- and 1.6-fold, respectively. This suggests that the chemical composition of the aerosol particles can play role in determining particle penetration through filter media. To further interpret the difference in penetration of MS2 virions and dextrose particles through the N95 swatch, we calculated the penetration values using the single fiber collection theory, accounting for diffusion and polarization forces (Lee and Mukund 2001; Lathrace and Fissan 1986; Lathrace et al. 1986), similar to the theoretical assessment approach and utilizing the same filter characteristics as described in Balazy et al. (2006b). It was assumed that the MS2 virion's dielectric constant was 55 (Aristides et al. 2007; Lepizco-Encinas and Rito-Palomares 2007), while that of dextrose was 3 (the dielectric constant of sucrose). Based

on these calculations, the expected penetration of particles through the N95 filter swatch was of 0.99% for dextrose and 0.70% for the MS2 virion- a ratio of 1.4; thus, the difference in particle penetration can be attributed, at least partially, to differences in particle dielectric constant. It is unclear as to why no difference was observed between the penetration of dextrose and bacteriophage particles through the N100 filter. From inspection, it was apparent that the N100 filter sample was electrically charged, however, the charged density was not known. It has been shown that for high efficiency filters particle collection by diffusion and by dielectrophoresis are not additive (Lee et al. 2002), as is assumed in conventional single fiber collection theory. For the N100 filters tested here, particle collection may occur primarily by diffusion only, thus the effects of particle chemical composition on particle penetration through these filters is minimal. In terms of using inert surrogate particles for testing filters and particle collectors for biological aerosols, these data show that not only must the physical size of biological aerosol particles and the inert surrogates be equivalent, but the dielectric properties must agree, if particle collection occurs by electrostatic means.

It is certainly possible to find appropriate surrogate particles. Despite the presence of contaminant particles and greater upstream count variability, nebulized MS2 aerosol particle filtration did not differ from that of the electrosprayed aerosol particles, regardless of the filter type. Nebulized contaminant particles are therefore suitable surrogates for clean virus particles, and nebulization of a bacteriophage MS2 suspension appears to be a robust method for physical bioaerosol filter testing over short-time durations under similar conditions to those used in the present study.

Virus viability

Both aerosolization techniques produced viable virus particles (Table 1). The higher titer and low dilution flow of the electrospray led to a viable virus aerosol particle concentration of ~3-fold greater (when using air) and ~20-fold greater (when using CO_2) than was obtained with the Collison nebulizer. The virus aerosol particle concentration was 95.2 PFU/cm^3 (plaque forming units per cubic centimeter) when generated by the nebulizer, 315 PFU/cm^3 when generated by the electrospray using dry filtered air, and 2,118 PFU/cm^3 when generated by the electrospray with CO_2. Use of air for aerosolization with the electrospray inhibited virus viability due to the production of reactive oxygen species. Direct comparison between aerosol generators can be made by defining a relative viability as the PFU/cm^3 in the generated aerosol divided by the PFU/mL originally in suspension, then normalizing the values such that the Collision nebulizer has a relative viability of 1.0. The electrospray with CO_2 as a sheath gas had a relative viability of ~4 times greater than the Collision nebulizer, while ~0.6 that of the nebulizer when air was used.

CONCLUSIONS

The utilization of a charge-reduced electrospray and nebulizer was examined for aerosolizing viruses under laboratory conditions to challenge respirator filters for their filtration efficiency testing. The electrospray is unique in its ability to produce single virion aerosol particles with low variability in the aerosolized particle concentration. However, it is a low-flowrate aerosol generator as compared to a nebulizer, and requires additional virus suspension preparation procedures for utilization. The data presented here can serve as general guidelines for the use of nebulizers and electrosprays for virus aerosol studies, including the capabilities and limitations of both aerosol generators.

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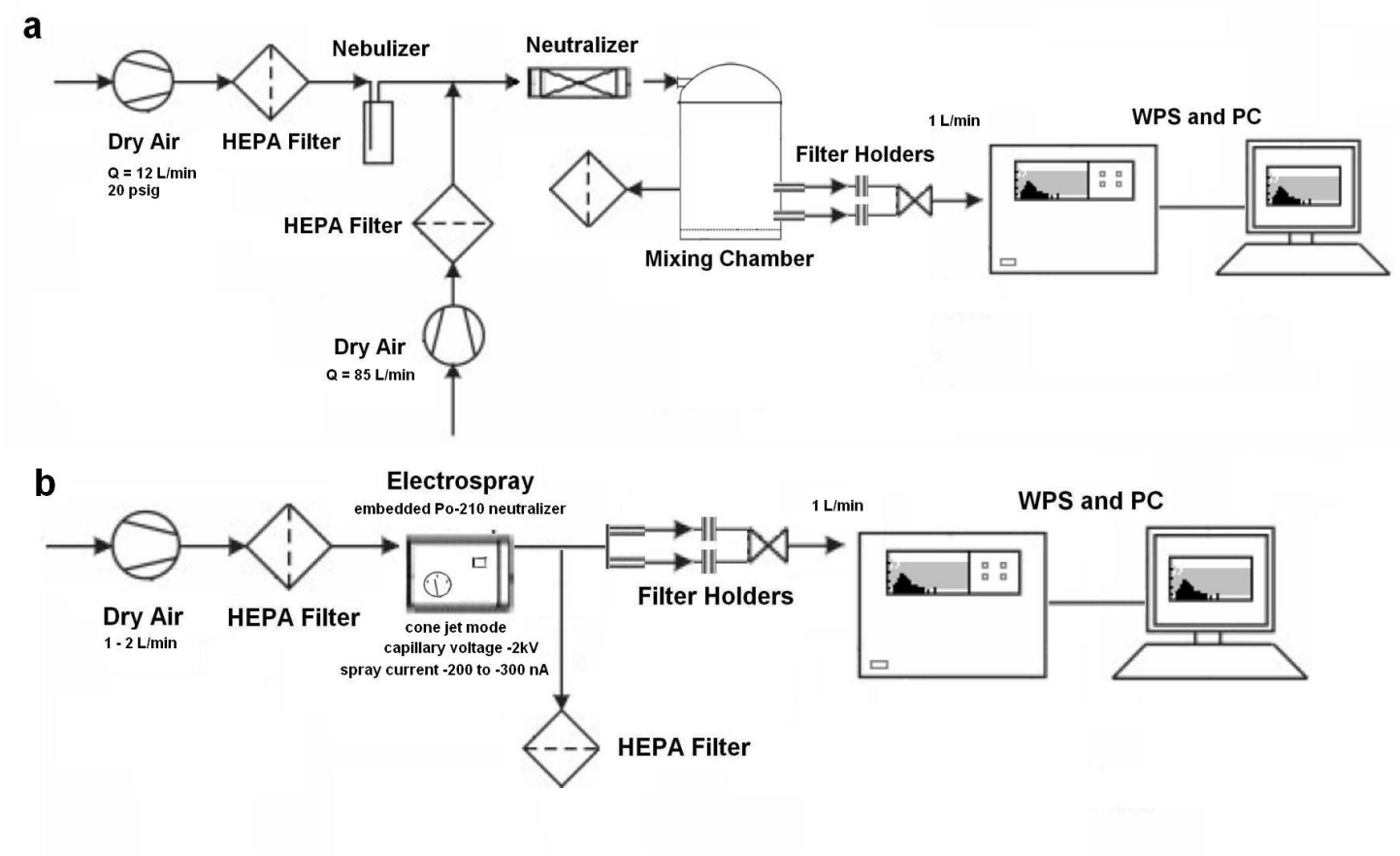

Figure 1. Experimental setups for filter testing: (a) experiments with nebulizer and (b) experiments with the electrospray.

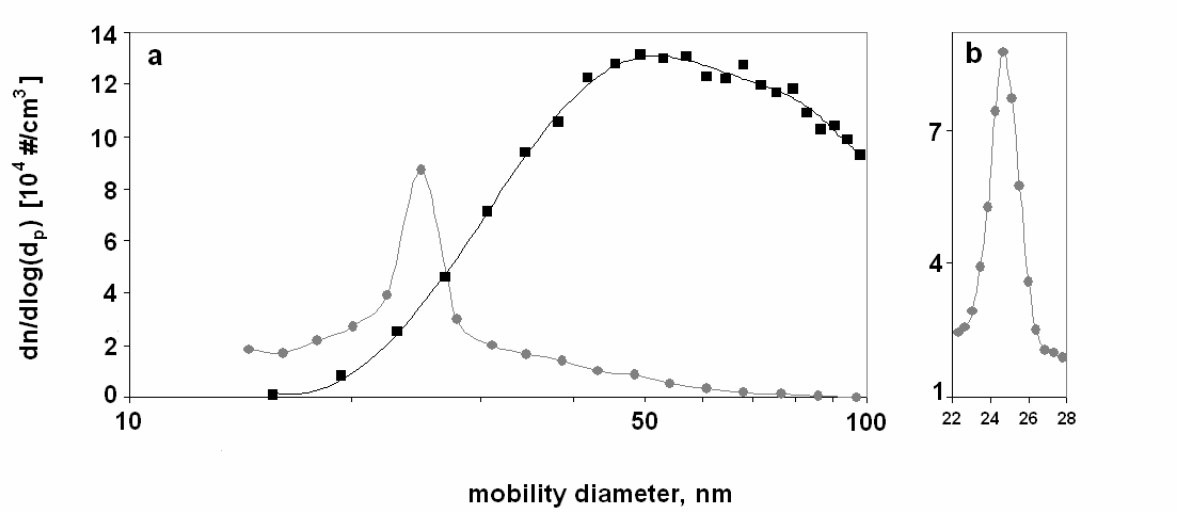

Figure 2. Bacteriophage MS2 aerosol particle size distribution for: (a) both aerosolization methods (nebulizer: black squares; electrospray: gray dots); (b) the electrosprayed size distribution in greater resolution, measured from 22 to 28 nm.

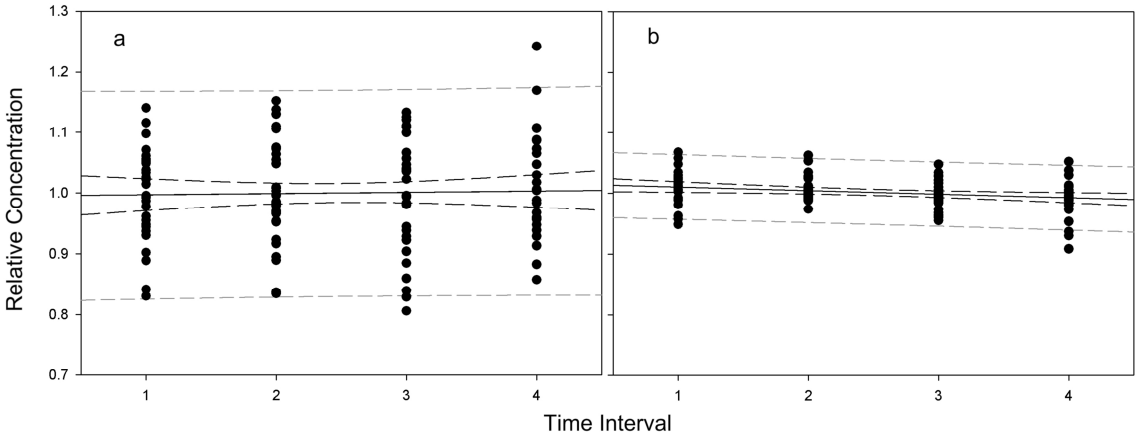

Figure 3. Variability of the relative particle concentration over repeated 15-minute filter tests. Shown as the upstream particle count from 23 – 26 nm normalized to the mean of each run for (a) Collison nebulizer; (b) electrospray.

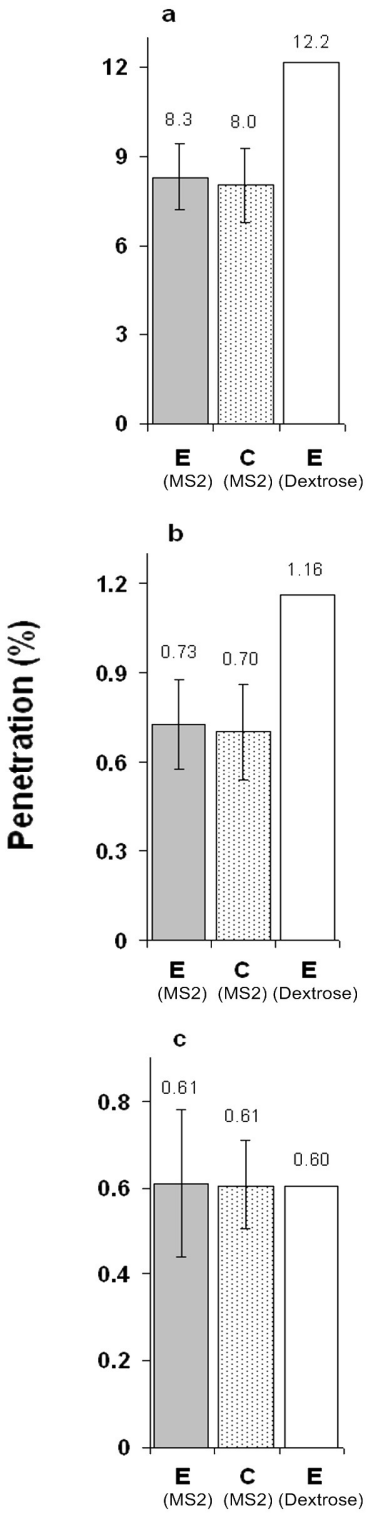

Figure 4. Particle penetration (±1 SD) for electrosprayed (**E**) bacteriophage MS2, Collison nebulized (**C**) bacteriophage MS2, and electrosprayed dextrose for each filter type: (a) surgical mask swatch; (b) N95 swatch; and (c) N100 swatch.

Table 1. Culturable Bacteriophage Comparison

Method	**Suspension Titer (PFU/mL)**	**Aerosol Concentration (PFU/cm^3_{air})**	**Relative Effectiveness[1]**
Nebulizer	2.2×10^9	95.2	1.0
Electrospray – air	1.2×10^{10}	315	0.6
Electrospray – CO_2	1.2×10^{10}	2118	4.0

[1] PFU/cm^3 in air per PFU/mL in the suspension, normalized to the nebulizer