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Respirator Testing Using Virus Aerosol: Comparison between Viability Penetration and Physical Penetration

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ABSTRACT

Viability, fluorescence (particle volume), photometric, viral RNA, and particle number penetration of MS2 bacteriophage through filter media used in three different models of respirators were compared to better understand the correlation between viability and physical penetration. Although viability and viral RNA penetration were better represented by particle volume penetration than particle number penetration, they were several-fold lower than photometric penetration, which was partially due to the difference in virus survival between upstream and downstream aerosol samples. Results suggest that the current NIOSH photometer-based test method can be used as a quick means to roughly differentiate respirators with different performance against virus aerosols.

KEYWORDS: airborne virus; filtration; particle volume penetration; photometric penetration; viability penetration

INTRODUCTION

Performance of filtering facepiece respirators against airborne viruses is often quantified in two ways. One is viability penetration (i.e. the percentage of infectious virus that penetrates through the respirator) and the other is physical penetration (i.e. the percentage of challenge particles/virus that penetrates through the respirator, regardless of viability) (Eninger *et al.*, 2008a). From an infection control perspective, measurement of the former, though labor-intensive, provides more valuable information than the latter.

Particle number penetration is the only form of physical penetration that has been compared with viability penetration (Eninger *et al.*, 2008a; Lore *et al.*, 2012; Booth *et al.*, 2013; Gardner *et al.*, 2013; Harnish *et al.*, 2013; Zuo *et al.*, 2013a). Without knowing the size distribution of infectious virus among polydisperse aerosol particles, however, such comparisons may be inappropriate (Gardner *et al.*, 2013). Recent studies (Zuo et al., 2013b, 2014) have shown that for a variety of viruses and nebulizer suspensions, infectious virus distribution in the size range of 100–450 nm generally follows particle volume distribution, suggesting that viability penetration may be particle volume-based instead of particle number-based. In addition, the current National Institute for Occupational Safety and Health (NIOSH) standard for respirator certification uses photometers to measure penetration (DHHS, 1995). It is not well understood

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how closely photometric penetration represents actual infectious virus penetration. Therefore, it is of interest to compare viability penetration with various physical measurements, particularly particle volume and photometric penetration.

METHODS

Filter media from three models of respirators labeled A, B, and C were tested. Models A and C were NIOSH approved N95 respirators while model B was not. Circular flat sheet filter media samples (16 cm diameter) of each model were clamped in a pneumatic chuck and evaluated using MS2 bacteriophage (ATCC 15597-B1) in an aerosol tunnel, which is a non-enveloped, icosahedron-shaped, and single-stranded RNA virus (Supplementary Figure S.1 is available at *Annals of Occupational Hygiene* online). Ideally, human/animal viruses should be used for the test. However, their low virus titer and poor airborne survivability, as well as high respirator filtration efficiency, made it difficult to recover viable virus downstream of the respirators. Therefore, they were excluded in this study. MS2 was aerosolized by a 6-jet Collison nebulizer at 10 psi from a suspension consisting of virus stock (4.5 ml), 3% tryptic soy broth (40.5 ml), and antifoam (0.1 ml). The distance between the nebulizer nozzle and the suspension level was maintained similar for each run to minimize variation in generated particle size distribution. Uranine (2 ml, 0.625 g ml^{-1}) was also added as a fluorescent particle tracer (Zuo et al., 2013b, 2014). After passing through a diffusion dryer and a Kr-85 neutralizer, the generated virus aerosol was mixed with HEPA-filtered room air and used to challenge the respirator at 85 l min^{-1}. Upstream and downstream concentrations were measured by a laser photometer (DustTrak II, TSI 8530) for particle light scattering and a scanning mobility particle sizer (SMPS, TSI 3034) for particle number distribution. In addition, samples were collected through upstream and downstream ports by two 25 mm diameter SKC gelatin filters at 2 l min^{-1} for 15 min. These filters were then analyzed by double agar layer plaque assay, quantitative RT-PCR (using QIAamp viral RNA kit), and spectrofluorometry for the amount of infectious virus, viral RNA, and fluorescence collected, respectively, as described elsewhere (Zuo et al., 2013b, 2014). Each test was repeated in triplicate using new samples of each model filter media at 10–20% relative humidity and 22–24°C.

Penetration was calculated as the concentration ratio downstream to upstream of the respirator for light scattering, infectious virus, viral RNA, fluorescence, and particle number. Assuming the amount of fluorescence carried per particle is proportional to the particle volume, the fluorescence penetration represents particle volume penetration. There might be difference between the two penetrations because uranine is highly hygroscopic, which changes particle volume with RH (Chan *et al.* 1997). Viability and physical penetration were statistically compared using one-way analysis of variance (ANOVA). In addition, relative recovery of infectious virus (RR_{IV}), an indicator for the survival of airborne viruses (Zuo et al., 2013b, 2014), was calculated by comparing the concentration ratio of infectious virus (C_{IV}) to fluorescence (C_F) in the gelatin filter (gel) and in the nebulizer suspension (neb): $RR_{IV} = (C_{IV,gel}/C_{F,gel})/(C_{IV,neb}/C_{F,neb})$.

RESULTS

Both upstream and downstream particle size distributions of MS2 aerosol were generally lognormal, with a count median diameter (CMD) of 86 nm and a geometric standard deviation (GSD) of 2.0 upstream and a GSD of 1.6 and a CMD of 70, 55, and 59 nm downstream for respirator models A, B, and C, respectively. Upstream viable virus and particle number concentration were 190–240 PFU cm^{-3} and 7.6×10^6–9.6×10^6 particles cm^{-3}, respectively, which were much higher than in natural environment (Verreault *et al.*, 2008). For all three models, viability and viral RNA penetration were closer to fluorescence (particle volume) and photometric penetration than particle number penetration (Table 1). In general, there was statistically significant difference between viability and physical penetration. Viability penetration was ~1/2 of viral RNA penetration, 1/5 to 1/3 of fluorescence (particle volume) penetration, and 1/10 to 1/5 of photometric penetration. RR_{IV} was found to be 1.04 ± 0.12 and 0.27 ± 0.06 ($n = 9$) for upstream and downstream aerosol samples, respectively.

To better compare the results, viability, fluorescence (particle volume), and viral RNA penetration were plotted versus photometric penetration for the three models of respirators (Fig. 1). There was a good linear correlation ($R^2 = 0.95$) between fluorescence (particle volume) penetration and photometric penetration. Viability and viral RNA penetration were also

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Table 1. Viability, fluorescence, photometric, viral RNA, and particle number penetration through different models of respirators tested at 85 L min^{-1}.

Respirator model	Penetration (%)							
	Viability	Fluorescence (Particle volume)[a]	*P* value[b]	Photometric	*P* value	Viral RNA	*P* value	Particle number All sizes[d]	*P* value
A	0.024 ± 0.004	0.12 ± 0.01	**<0.001**[c]	0.23 ± 0.01	**<0.001**	0.051 ± 0.006	**0.003**	3.40 ± 0.57	**<0.001**
B	0.008 ± 0.003	0.029 ± 0.004	**0.001**	0.040 ± 0.007	**0.002**	0.016 ± 0.004	0.054	1.97 ± 0.41	**0.001**
C	0.014 ± 0.004	0.042 ± 0.007	**0.003**	0.070 ± 0.015	**0.003**	0.024 ± 0.008	0.117	2.06 ± 0.47	**0.002**

Data are reported as mean ± 1 SD ($n = 3$).
[a]Fluorescence penetration represents particle volume penetration, assuming that the amount of fluorescence carried by a particle is proportional to the particle volume.
[b]For each respirator model, one-way ANOVA compares viability penetration and one form of physical penetration.
[c]Values in bold font are *P* value < 0.05.
[d]Assumes lognormal particle size distributions for aerosol samples both upstream and downstream of the respirators. SMPS size range: 10–470 nm.

Figure 1 Correlations between photometric penetration and fluorescence, viability, and viral RNA penetration. The solid line indicates a 1:1 correspondence between the two axes.

reasonably correlated with photometric penetration, following a power-law relationship.

DISCUSSION

Virus-containing particle size distributions produced by Collison nebulizers generally follow particle volume distribution, not particle number distribution (Zuo et al., 2013b, 2014), which made us hypothesize that particle volume penetration might represent viability penetration through respirators. In this study, viability penetration was indeed found to be better represented by particle volume penetration than particle number penetration (Table 1). However, viability penetration was still significantly different from fluorescence (particle volume) penetration ($P < 0.05$). The exact cause of this difference remains unknown, but it may be partially attributed to the different survivability of airborne MS2 between upstream and downstream samples. As indicated by the higher upstream than downstream RR_{IV} values, the virus aerosol upstream survived better (i.e. maintained its viability) and was thus recovered more efficiently than downstream. Since fluorescence (particle volume) was detected with similar efficiency upstream and downstream, viability penetration was therefore lower than particle volume penetration. Large particle sizes can possibly enhance virus survival due to the shielding effect. Namely, compared with small particles, virus carried by larger particles is surrounded by

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more solute in the nebulizer suspension, which forms a shield and better protects the virus from environmental stress (Woo *et al.*, 2012; Zuo *et al.*, 2013b). During the tests, aerosol particles upstream were generally larger (e.g. larger CMD) than downstream, which may be the reason for the higher virus survival upstream than downstream. A similar situation might exist for viral RNA (Zuo *et al.*, 2013b), but because it is more stable than virus viability in air, viral RNA penetration was higher than viability penetration. Since the survival of airborne virus affects the determination of viability penetration, test protocols for respirators challenged with virus aerosols [e.g. particle size distribution, environmental conditions (Lore *et al.*, 2012), and composition of the nebulizer suspension (Zuo *et al.*, 2014)] should be well documented and standardized.

Similar to fluorescence penetration, photometric penetration is also a particle volume-based penetration measurement. Photometry is a conventional way to estimate aerosol mass. However, photometers are not sensitive to particles <100 nm (Eninger *et al.*, 2008b; Rengasamy and Eimer, 2012) and therefore the measured photometric penetration represents that of larger size particles, which might be one of the reasons for the discrepancy between fluorescence and photometric penetration. Nevertheless, few studies have compared photometric and viability penetration. It was found that viability penetration was lower than expected when compared to photometric penetration, even after taking RR_{IV} into account, suggesting that the photometer-based test method, similar to the NIOSH standard for respirator certification, provides a conservative estimate for respirator performance against virus aerosols. In addition, both the photometer-based test and virus viability-based test ranked respirators in a similar and predictable manner (Fig. 1), indicating that the photometer-based test can also be used as a quick but rough method to differentiate respirators with different virus infection control capabilities.

In general, penetration of virus aerosol through respirators depends on not only the challenge aerosol size distribution, the particle size dependent penetration of the respirator, and how each instrument detects and measures aerosol/virus (Biermann and Bergman, 1988; Li *et al.*, 2012), but also virus survival and virus content distribution in the challenge aerosol particles, as demonstrated in this study. In addition, different virus type (e.g. bacteriophage versus animal/human viruses) may have significantly different penetration. For future work, these parameters should be fully understood in order to predict viability penetration based on physical penetration measurements.

SUPPLEMENTARY DATA

Supplementary data can be found at http://annhyg.oxfordjournals.org/.

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DISCLAIMER

The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of CDC- NIOSH.

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