Development and Characterization of a New Test System to Challenge Personal Protective Equipment with Virus-Containing Particles

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ABSTRACT

Previous bioaerosol test systems used in personal protective equipment (PPE) reuse, performance, and handling research were limited in their ability to generate diverse particle size ranges and types. The objective of this study was to develop and characterize a new test system to challenge PPE with virus-containing particles (VCPs). The new system was designed to achieve two specific research objectives: 1) to be capable of delivering VCPs uniformly onto air permeable PPE such as filtering facepiece respirators (FFRs) and surgical masks (SMs) and 2) to be capable of performing simple VCP filtration tests. The test system consists of two aerosol generators, an exposure chamber, a breathing simulator/head form, and several aerosol detection systems.

The test system was validated against the two objectives using two experimental scenarios involving droplet nuclei and droplet VCPs. The size distribution from the droplet nuclei experiments was 0.02 - 10.3 μm, with 96% of particles between 0.2 - 4.0 μm and a mass median diameter of 0.60 μm with a geometric standard deviation (GSD) of 1.64. The size distribution of the droplets was 0.54 - 100 μm, with 83% of particles < 10 □m and a median [Dv(50)] of 5.03 □m. The amount of viable MS2 deposited on the respirators met ASTM E2720 and E2721 loading requirements, with > 97% found on the outer and middle layers of the N95 FFR models. Average filtration efficiencies were highest for the P100 FFRs (99.91 - 99.94%), followed by N95 FFRs (96.57 - 98.18%) and SMs (78.69 - 80.43%). These data indicate that the test system was able to meet the study objectives and will serve as a versatile tool for standards development and for research studies related to PPE reuse and handling.

Keywords: Bio-aerosol respirator testing system, viral droplets, droplet nuclei, loading density, uniformity, filtration efficiency

INTRODUCTION

Virus-containing particles (VCPs) produced by talking, breathing, coughing, and sneezing span a diversity of sizes and contribute to the spread of some diseases (IOM, 2011). According to the Centers for Disease Control and Prevention (CDC), respiratory viruses such as influenza may be transmitted among humans in three ways: 1) direct-contact which involves transport of the virus from virus-contaminated hands or objects (e.g., fomites) to the mucous membranes (e.g., by touching the face); 2) droplet-spray transmission occurs when large VCPs (> 100 μm aerodynamic diameter) are expelled by an infected person, travel a short distance through the air, and deposit immediately onto the

mucous membranes of another person; and 3) aerosol transmission which occurs via inhalation of VCPs (Snider, 2010; IOM, 2011). These particles can be classified as respirable or inspirable (Nicas and Sun, 2006; Jones and Adida, 2011), based upon where they deposit in the respiratory tract. Respirable particles (< 10 µm aerodynamic diameter) remain airborne sufficiently long to provide a mechanism for airborne transmission, while inspirable particles (10–100 µm aerodynamic diameters) are either inhaled at close contact or they gravitationally settle very fast. For simplicity, in this paper, the term "droplet nuclei" will refer to dry particles, in the respirable and inspirable size ranges, that have evaporated, while the term "droplet" will refer to wet particles that have not completely evaporated yet, regardless of the particle size.

Personal protective equipment (PPE) such as filtering facepiece respirators (FFRs) and surgical masks (SMs) are often used as non-pharmaceutical interventions to reduce the spread of respiratory viruses. When used in the presence of an infected patient, PPE can become contaminated with VCPs and may serve as a fomite. Thus, PPE are typically discarded after each patient-encounter during a pandemic situation, resulting in a need for research to develop appropriate strategies for handling contaminated PPE and for PPE reuse during shortages (IOM, 2006; IOM, 2011).

Recently, several test systems have been developed for contamination of FFRs by deposition of VCPs (Fisher et al., 2009; Heimbuch et al., 2011; Vo et al., 2009; Woo et al., 2010). The bioaerosol respirator test system (BARTS) of Fisher et al. (2009) was designed to provide viral droplet nuclei with a particle size <1 µm for loading onto FFR coupons. The droplet phase aerosol respirator testing system (DPARTS) of Vo et al. (2009) was targeted toward generating viral droplets. The systems used by Woo et al. (2010) and Heimbuch et al. (2011) were also focused on specific aerosol size ranges and types (droplet or droplet nuclei), using either gravity or constant air flow to deliver the viral aerosol to the FFR. Development of a single test system, which can reproducibly generate a diverse range of both virus-containing droplets and droplet nuclei and deliver them uniformly onto PPE under simulated human breathing waveforms, would provide a valuable new tool for studying appropriate handling of contaminated PPE and PPE decontamination/reuse.

The aim of the present study was to develop and characterize a new test system to challenge PPE with VCPs. The new system was designed to achieve two specific research objectives: 1) to be capable of delivering a diverse range of both virus-containing droplets and droplet nuclei uniformly onto PPE and 2) to be capable of performing simple VCP filtration tests of PPE. To validate the performance of the test system against the two criteria, two experimental situations were chosen: one involving a polydispersed distribution of "dry" respirable and inspirable-sized VCPs (i.e., "droplet nuclei") and the second involving a polydispersed distribution of larger presumably "wet" VCPs ("droplets") spanning both the inspirable and respirable size ranges.

MATERIALS AND METHODS

Preparation of MS2 virus, plaque assay, and particle generator fluid

***Preparation of MS2 virus*.** The bacterial strain, *Escherichia coli* (*E. coli*, ATCC 15597) and bacteriophage-MS2 (ATCC 15597-B1) were obtained from the American Type Culture Collection (ATCC; www.atcc.org). A culture medium (271B) and MS2 were prepared according to the method of Vo et al. (2009). The final MS2 suspension [10^{11} plaque forming units per milliliter (PFU/mL)] was obtained, and designated as a stock MS2 suspension. MS2 was selected for the study based on its survivability, ease of preparation, and non-pathogenicity (Jones et al., 1991). An option to use MS2 was also included in the ASTM methods as a challenge bioaerosol (ASTM E2720, 2010; ASTM E2721, 2010).

Plaque assay. An overlay agar assay method was used to enumerate the viruses. Sterile glass tubes containing 0.1 mL of overnight *E. coli* and 0.1 mL of the diluted MS2 were warmed in a water bath at 45 °C. A 3-mL volume of melted soft agar (0.5% agar) was added to each tube and mixed thoroughly before pouring it into a labeled Petri plate containing hard agar (1.5% agar). The plates were covered and the agar was allowed to gel. The plates were inverted and incubated at 37 °C overnight. The plaques were counted, multiplied by the dilution factor, and divided by the sample volume to obtain the titer in PFU/mL.

Particle generator fluid. All MS2-containing suspension solutions (aerosol generator fluid) were prepared by diluting the stock MS2 suspension into 271B medium to the final suspension concentration of approximately 10^7 PFU/mL. This concentration level was chosen to ensure adequate MS2 detection on the inner layer of the test FFR described in the "PPE loading (contamination) procedure" section. The density of this homogeneous fluid is approximately 1.01 g/mL based on the known mass and volume of all components in the mixture.

Equipment and supplies

Bio-aerosol respirator testing system. The custom designed bio-aerosol respirator testing system (BARTS-II) is shown in Fig. 1, and consists of four major components. 1) Particle generation, consisting of a six-jet Collison nebulizer (BGI, Waltham, MA; to generate small droplets) and a Vibrating Orifice Aerosol Generator (VOAG; TSI, Shoreview, MN; to generate large droplets). A compressed air supply for both generators was filtered with a high efficiency particulate air (HEPA) filter. 2) An 800-L exposure chamber, including a humidity/temperature sensor, circulation fans, a humidity/temperature controller, an Ultrasonic Humidification System with a nitrogen gas supply, and a 9.0-cm diameter exhaust port, containing a HEPA filter and an internal fan to direct air flow (Electro-Tech System, Glenside, PA). The width, depth, and height of the exposure chamber are 1.0 m x 1.0 m x 0.8 m, respectively so a head form could be placed inside the chamber and easily disassembled for sterilization. Because of bioaerosol safety concerns, the exposure chamber was set up inside a secondary acrylic containment chamber (1.2 m x 1.2 m x 1.0 m as width, depth, and height, respectively; Vandiver Enterprises, Zelienople, PA). Air ventilation was used between the chambers to prevent contamination. 3) A breathing system that used a plaster-material head form and Series 1101 breathing simulator (Hans Rudolph, Shawnee, KS). 4) A detector system, consisting of a Scanning Mobility Particle Sizer (SMPS; model 3080; TSI; detection range: 0.01–1 □m), an Aerodynamic Particle Sizer (APS, Model 3321, TSI; detection range: 0.5–20 □m), and a Spraytec laser diffraction system (Model STP5315, Malvern Instruments, Inc., Westborough, MA) with an inhalation cell to provide an enclosed measurement zone under a vacuum flow rate of 20 L/min (Malvern Instruments, Westborough, MA; detection range: 0.1–1000 □m).

Breathing system. The breathing system shown in Fig. 1 was used to load VCPs onto PPE during simulated human breathing. A full FFR or SM (depending upon the experiment), sealed by silicone sealant to the face of the head form, was located at a distance of 32 cm from the particle outlet inside the chamber to simulate very close contact between a healthcare worker and an infected patient. The head form was connected to a breathing simulator using a plastic breathing tube (Fig. 1, 5A and 5B). Air was inhaled and exhaled through the breathing tube with the sequence being controlled by the breathing simulator to generate a breathing waveform of 30 L/min (1.2 L/stroke x 25 strokes/min). The breathing waveform employed in this study represents a normal work rate from a workplace scenario (Clayton et al., 2002). Before each breathing experiment, the silicone sealant surface was covered by a bubble-producing liquid to determine if the exhaled air caused bubble formation in case of a leak, and if any leaks were detected, additional silicone was applied to the seal and the leak check repeated.

N95/P100 FFRs and SMs. Three different models of FFRs approved by the National Institute for Occupational Safety and Health (NIOSH) and one SM model were selected for this study (Table I). Each FFR and SM model has different characteristics, such as number of layers and different hydrophilic and hydrophobic materials.

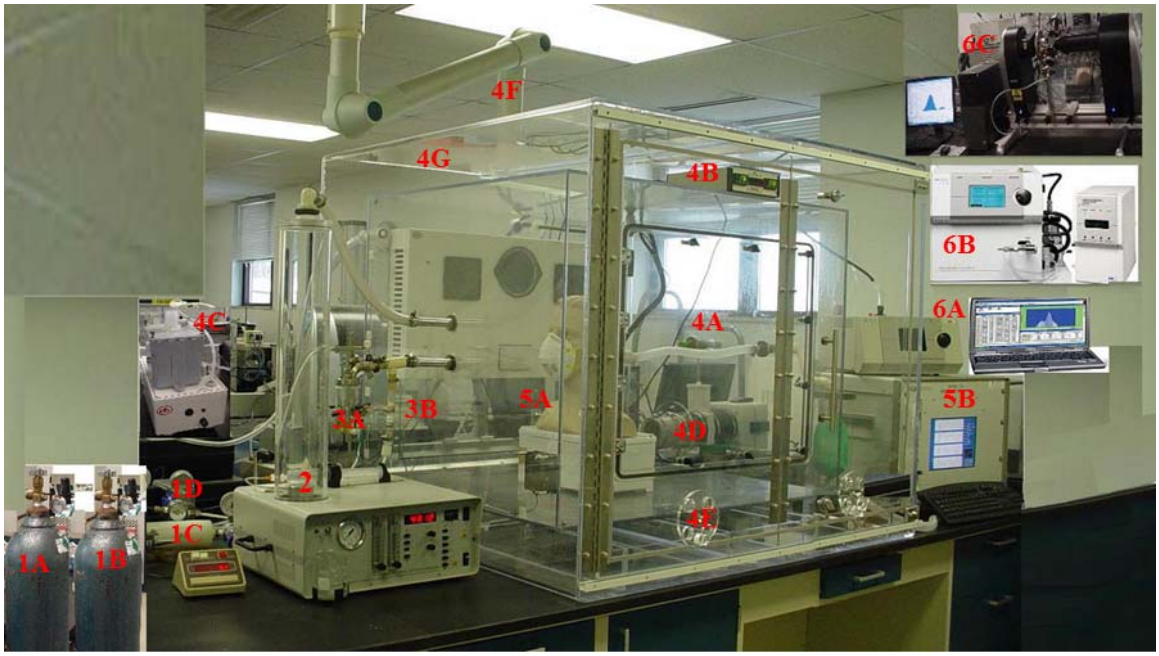

Fig. 1. The bio-aerosol respirator testing system: compressed air supply (1A); nitrogen air supply (1B); HEPA filter (1C); air flow regulator (1D); VOAG (2); nebulizer (3A); nebulizer dilution air inlet (3B); exposure chamber (4A); temperature and RH controller (4B); Ultrasonic Humidification generator (4C); exhaust port with HEPA filter (4D); ventilation air inlet (4E); ventilation air outlet (4F); a secondary containment (4G); a head form with a FFR (5A); breathing simulator (5B); APS system (6A); SMPS system (6B); and Spraytec Laser system (6C).

Aerosol generation and PPE loading procedures

Generation of MS2 droplet nuclei. The MS2 suspension solution (45 mL) was added to the nebulizer glass jar and the VOAG syringe for each exposure test. After the chamber was sealed and the desired temperature and RH were achieved (23 ºC, 35% RH), the chamber was operated for 5 min, while running the humidity/temperature controller, fans, and Ultrasonic Humidification System to ensure stability of chamber conditions. Then, compressed air valves were opened, allowing air flow through the nebulizer and VOAG. The nebulizer and VOAG were run simultaneously to generate MS2 droplet nuclei. For the nebulizer, the airflow was set to 20 psi (12 L/min; volumetric MS2 suspension leaving the nebulizer at approximately 0.22 mL/min) and the viral particles were directly mixed with HEPA-filtered dry dilution air (30 L/min; Fig. 1, 3A and 3B). For the VOAG, a 20-µm orifice with a frequency in the range of 40-80 kHz was used. The airflow to the VOAG was set to 30 psi (1 L/min as a dispersion air and 49 L/min as a carrier/dilution air) and the volumetric MS2 suspension leaving the VOAG was 0.14 mL/min. The exhaust port was in the open position during particle generation and sampling to remove excess air. During aerosolization, droplet nuclei particles (including MS2 virus, impurities, other constituents of the generator fluid, etc.) were continuously dispersed into the chamber. Given the low humidity in the chamber and high dilution air mixed with the aerosol stream, particles formed droplet nuclei in the chamber via evaporation. For simplicity, aerosol generated using this process was designated as “MS2 droplet nuclei”.

TABLE I. PPE Characteristics

FFR/ surgical mask type	FFR/surgical mask information	Layer Number and Materials
Willson P100 FFR	Model: Willson P1130 M/L Manufacturer: Willson Santa Ana, CA 92704 Retail: www.labplanet.com 1-800-504-7309	Three layers: Layer 1: thin hydrophobic materials (outer-layer) Layer 2: thick hydrophobic materials (middle layer) Layer 3: thin hydrophobic materials (innermost-layer)
North N95 FFR	Model: North 7130N95 Manufacturer: www.northsafety.com Cranston, RI 02921 1-800-430-4110	Four layers: 1: thin hydrophobic materials (outer-layer) Layer 2: combination of thin hydrophobic and hydrophilic materials (middle layer #1) Layer 3: thick hydrophobic materials (middle layer #2) Layer 4: thin hydrophilic materials (innermost-layer)
Gerson N95 FFR	Model: Gerson 1730 Manufacturer: www.GersonCo.com Middleboro, MA 25-8623 Retail: www.masksnmore.com 1733 S. Fretz Ave. Suite B Edmond, OK 73013	Three layers: hydrophilic materials (outer-layer) Layer 2: hydrophobic materials (middle-layer) hydrophilic materials (innermost-layer)
Surgical mask	Model: 3M 1800 Manufacturer: 3M Retail: www.healthykin.com	Two layers: Layer 1: very thin hydrophobic materials (outer-layer) Layer 2: very thin hydrophobic materials (inner-layer)

Generation of MS2 droplets. MS2 was loaded into the aerosol generators as described for the droplet nuclei experiments. After the chamber was sealed and the designated temperature and RH were reached (25 ºC, 90% RH), compressed air valves were opened and viral droplets were continuously generated during the experiment (for the nebulizer: no dilution air; for the VOAG: 1 L/min as a dispersion air, 29 L/min as a carrier air, and a 50-μm orifice with the frequency in the range of 8-20 kHz were used). Given the high humidity level in the chamber and the lack of dilution air, VCPs and other particles generated during this aerosol generation process are presumably "wet" (i.e., less drying due to evaporation) and designated as "MS2 droplets" for simplicity.

PPE loading (contamination) procedure. In the development of the loading procedure, the need for sufficient viral particles was considered to permit adequate viable detection. Bioassay test samples were diluted appropriately and plated to show 30 – 300 PFU/plate to ensure acceptable data quality (*APHA*, 1998). Based on the bioassay technique, the loading time, and the extraction process (re-suspending viruses from the sample), the minimum detection limit (MDL) required a minimal loading concentration (MLC) of 750 PFU/cm^2 (based on: minimal viral detection of 30 PFU/plate; 0.1 mL of extraction solution used for bioassay; 10 mL of 271 B medium used to extract each 4-cm^2 tested filter). Thus, the suspension concentration (aerosol generator fluid) had to be at least 3x10^4 PFU/mL (based on the back calculation of equation 1, staring with the MLC of 750 PFU/cm^2) to achieve the MLC for the tested FFRs; however, for quantifying viable MS2 trapped within each layer of FFR, the suspension concentration had to be approximately 10^7 PFU/mL to ensure adequate MS2 for detection on the inner layer of the test FFR.

When the exposure chamber and the breathing system were set up, VCPs were generated and a timer was started to measure the loading duration. VCPs were subsequently loaded onto the PPE using the breathing simulator for 22 min. With the average area of each PPE item being tested ~148 cm^2 (excluding the area sealed by silicone to the face of the head form), the theoretical MS2 loading concentration (C_{TLC}) was *calculated* using Equation 1 and found to be 2.18 x 10^5 PFU/cm^2.

$$C_{TLC} = \frac{C_{Average} \times B_{BW} \times T_{Loading}}{A_{FFR}} \qquad \text{(eq. 1)}$$

where $C_{Average}$ is average chamber concentration, 49 PFU/mL (generator fluid, 10^7 PFU/mL; suspension leaving nebulizer and VOAG, 0.359 mL/min; generating time, 22 min; chamber volume, 800 L); B_{BW} is breathing waveform, 3x10^4 mL/min; $T_{Loading}$ is loading time, 22 min; A_{FFR} is FFR area, 148 cm^2.

Once the MS2 particle loading was completed, the air supply, generators, and the chamber operation were stopped, and the exposed PPE item being tested was retrieved and saved for viable MS2 measurement.

Aerosol characterization experiments

Size distribution of MS2 droplet nuclei (at the headform). The size distribution and concentration of the MS2 droplet nuclei outside the FFR (near the center) were measured using an APS with a 90-cm probe connecting the test FFR to the APS (Fig. 1, 6A) and an SMPS, including a condensation particle counter (CPC, model 3025A; TSI) under a controlled flow rate of 1.5 L/min with another 90-cm probe connecting the test FFR to the SMPS (Fig. 1, 6B). The probes connecting the FFR to the particle detectors were only used for the particle size distribution and the chamber uniformity experiments. The combination of the SMPS and APS data into a single size distribution (0.02 – 20 µm) was performed according to the method of Khlystov et al. (2004) by calculating the ratio of the overlapping size range between 0.6 and 0.9 µm.

Size distribution of MS2 droplets (at the headform). The size distribution and concentration of the MS2 droplets outside the FFR (near the center) were measured using the Spraytec with a 90-cm probe connected (Fig. 1, 6C).

Chamber uniformity experiments. In addition to the main (built-in) humidity/temperature controller in the exposure chamber, a humidity/temperature TESTO 635-1 (www.testo.com) was also employed to investigate the RH and temperature at different chamber locations designated as: back-left top (BLT), front-left top (FLT), back-right top (BRT), front-right top (FRT), center (C), back-left bottom (BLB), front-left bottom (FLB), back-right bottom (BRB), and front-right bottom (FRB). The SMPS and Spraytec were also used to characterize the particle size distribution at these locations.

PPE contamination experiments

Determination of MS2 survival under experimental conditions. Before quantifying viable MS2 deposited onto PPE, the MS2 viability during the particle generation and the loading period was examined to determine if these experimental conditions would interfere with the survival of MS2 virus. To evaluate how MS2 viability was influenced by the nebulizer/VOAG generation and to estimate other sources of possible particle losses, MS2 particles on a P100 FFR were collected and their viability compared with the theoretical MS2 loading concentration. A P100 FFR model was selected for this experiment because of its high efficiency for capturing submicron particles**.** The MS2 survival on the P100 FFR during the loading period was also examined by applying MS2 onto the FFRs for 2 min and then storing the MS2 contaminated FFR samples for 1, 7, 14, and 22 min (at the same loading conditions). Three replicate tests were carried out for each set of conditions. The exposed P100 FFRs were removed from the head form and cut into 4-cm^2 (2 cm x 2 cm) coupons with scissors, subsequently using forceps to place each coupon in 10.0 mL of 271B medium in a 50-mL conical tube for extraction. MS2 was extracted by agitating with a vortex mixer set on high for 2 min. Then, the coupons were discarded and the supernatant was assayed for viable MS2.

The extraction efficiency from the filter media was also determined according to the method of Vo (Vo *et al*. 1999) as follows. The same volumes of MS2-containing suspension solutions were spiked: 1) onto the surface of the filter media before dipping them into 10 mL of 271B medium in a 50-mL conical tube as test samples and 2) directly into 10 mL of 271B medium in a 50-mL conical tube as a control. These conical tubes were then used for extraction and extraction efficiency analysis.

PPE contamination variability. To investigate the uniform (spatial) deposition of VCPs, viable MS2 trapped at different locations (top, center, bottom, left, and right areas; Fig. 2) were measured after a 22-min loading period using the breathing simulator. The contaminated FFR was removed from the chamber and cut into 4-cm^2 coupons, with each coupon being placed in 10.0 mL of 271B medium in a 50-mL conical tube for the extraction and a plaque assay process. For the North and Gerson N95 FFRs, the amount of viable MS2 trapped within each layer was determined by separating each layer prior to cutting into 4-cm^2 coupons. The data were analyzed to determine if the test system met the quality requirements for loading specified in ASTM E2720-10 and E2721-10 which specify a coefficient of variation (CV) within a given sample (location to location or L-T-L) and across independent samples (sample to sample or S-T-S) of less than 20% and 40%, respectively.

Filtration efficiency against MS2 containing particles

For filtration testing, the challenge of the PPE was performed similar to the protocol described above; however, in this procedure, two additional Willson P100 FFR samples (3.2-cm diameter circular coupons) were used for each experiment to quantify the number of viable MS2 that passed through the PPE being tested. These secondary P100 FFR samples were designated as “downstream filters.” Two downstream filters were placed in both ends of a sample holder (the front: 2A and the back: 2B) located at the end of the plastic tube in the back of the head form’s neck (Fig. 2). The sample holder was connected between the head form and the breathing simulator. When the breathing system was started, VCPs in the chamber interacted with the PPE sealed to the head form. Particles that passed through the PPE could be trapped on the first downstream filter. If any particles passed through the first downstream filter, they could be trapped on the second downstream filter.

The exposed PPE and downstream filters were cut into small coupons (4.00-cm^2 for FFRs/surgical masks and 2.54-cm diameter for the downstream filters) and each coupon (all layers) was then placed in 10 mL of 271B medium in a 50-mL conical tube for extraction. Upon completion of the extraction process, viable MS2 trapped throughout the PPE and the downstream filters were measured.

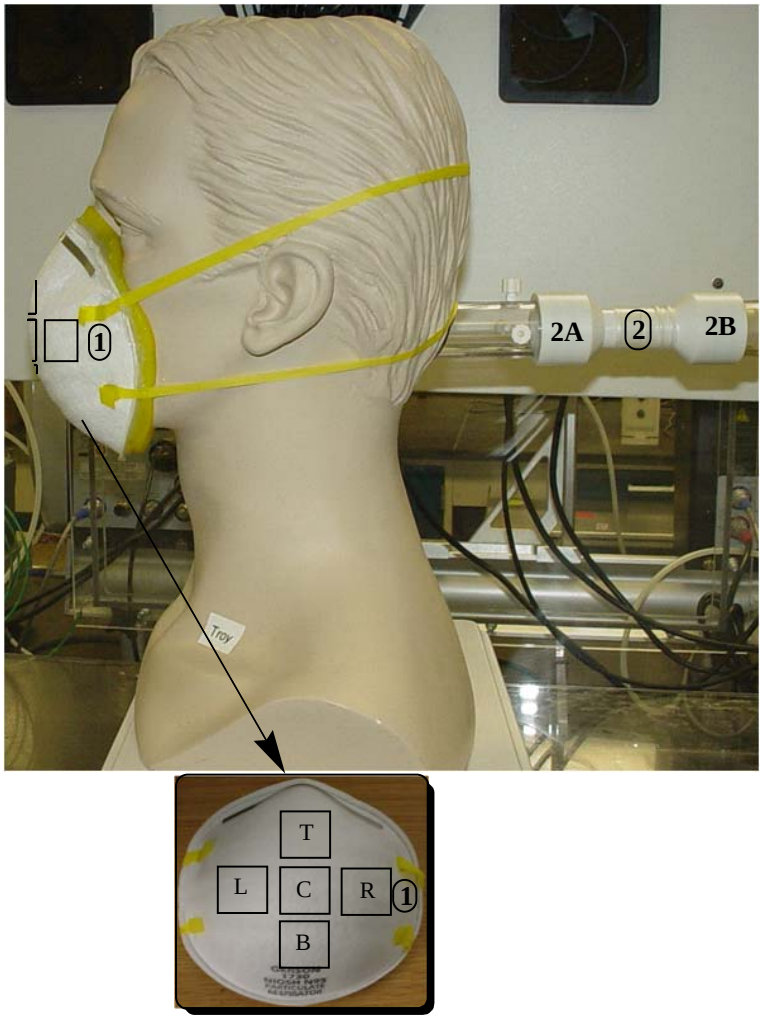

Fig. 2. Head form and downstream collection filters: test FFR (1); sample coupons on the FFR for the uniform deposition experiment (T, top; C, center; B, bottom, L, left; R, right); downstream collection filters' holder for the filtration efficiency experiment (2); the first downstream collection filter in the front filter holder (2A) and the second downstream collection filter in the back filter holder (2B).

Conventional measurements of filtration efficiency are based on the ratio of the particle concentrations upstream and downstream of the device being tested (Balazy et al., 2006). In this study, filtration efficiency (FE) was measured based on the number of viable viruses captured (retained) by the PPE being tested and the number of viable viruses that penetrated the PPE. FE was calculated as:

$$FE = [1 - (N_p/N_e)] \times 100 \qquad \text{(eq. 2)}$$

where N_p is the number of viable MS2 that penetrated the PPE (i.e., MS2 trapped on the downstream filters; PFU/cm^2/L); N_e is the number of viable MS2 challenging the PPE (MS2 recovered from the PPE being tested + MS2 recovered from the downstream filters).

RESULTS

Aerosol characterization experiments

The size distribution of the particles in the droplet nuclei experiments, measured using the SMPS and APS, ranged from 0.02 to 10.3 µm, with 96% of particles centered between 0.2–4.0 µm (Fig. 3). The mass median diameter (MMD) was 0.60 □m with a geometric standard deviation (GSD) of 1.64 and a mode of 0.96 □m (Fig. 3). As shown in Figure 4, the size distribution of the particles in the droplet experiments, measured using the Spraytec, ranged from 0.54 to 100 □m. Most (83%) of the particles in the droplet experiments were < 10 □m, with a median size [Dv(50)] of 5.03 □m.

Figure 5 shows the size distribution of the aerosol generated during the droplet nuclei experiments at different chamber locations as measured using the SMPS and APS. The average concentrations of particles in the droplet nuclei experiments ranged from 1.72–1.82 x 10^7 particles/cm^3 (n=3). All CVs at different chamber locations were found to be ≤ 1.93%. The average RH and temperature for these experiments were found to be 35.11% RH and 23.17 ºC with a standard deviation (σ) of 0.37 and 0.14, respectively. The average concentrations of particles in the MS2 droplet experiments were found to be from 6.39–6.90 x 10^4 particles/cm^3 (n=3), with CVs at different chamber locations ≤ 2.65%. The average RH and temperature were found to be 90.14% RH and 25.24 ºC, with an σ of 0.39 and 0.12, respectively.

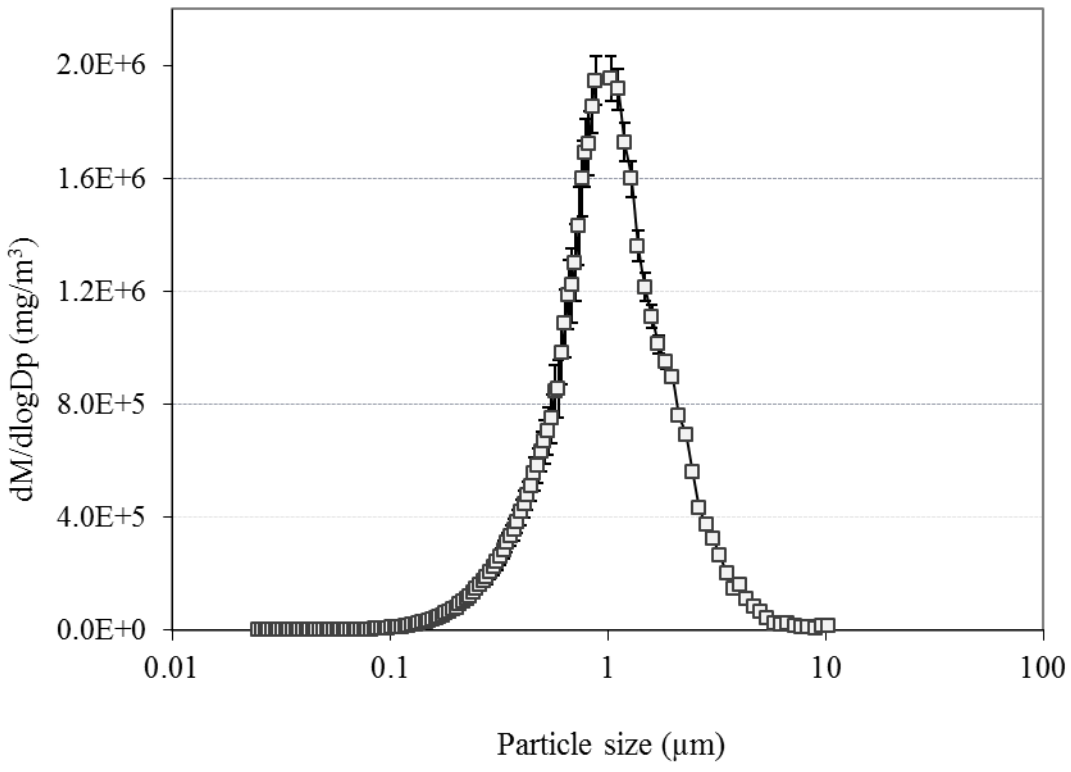

Fig. 3. Size distribution of the aerosol from the droplet nuclei experiments measured at the headform using the APS and SMPS (combined).

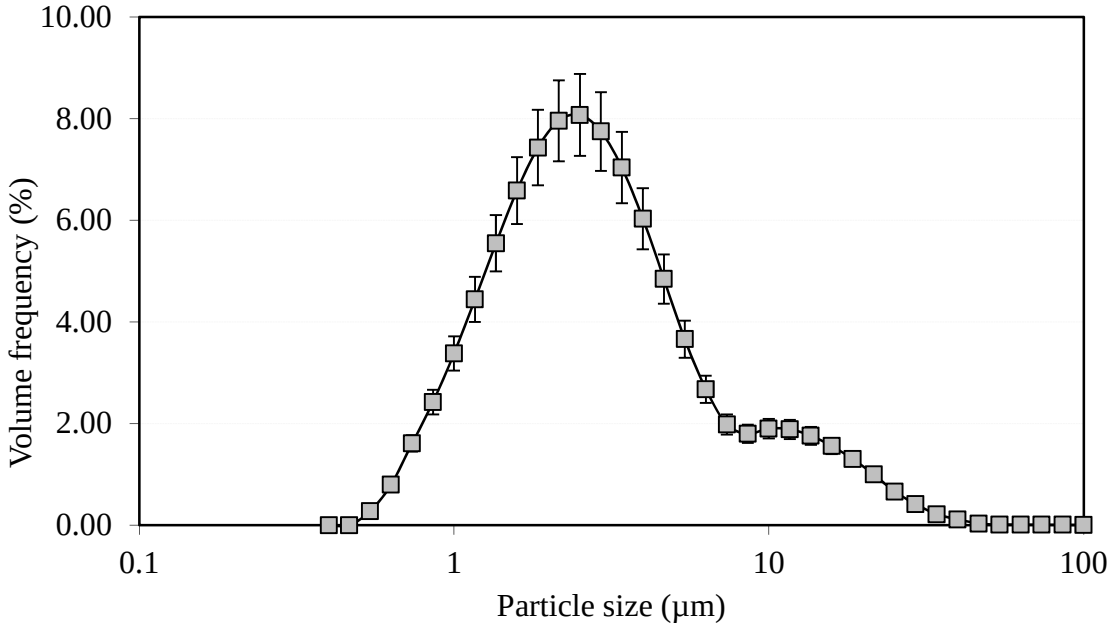

Fig. 4. Size distribution of the aerosol from the droplet experiments measured at the headform using the Spraytec laser diffraction particle size system.

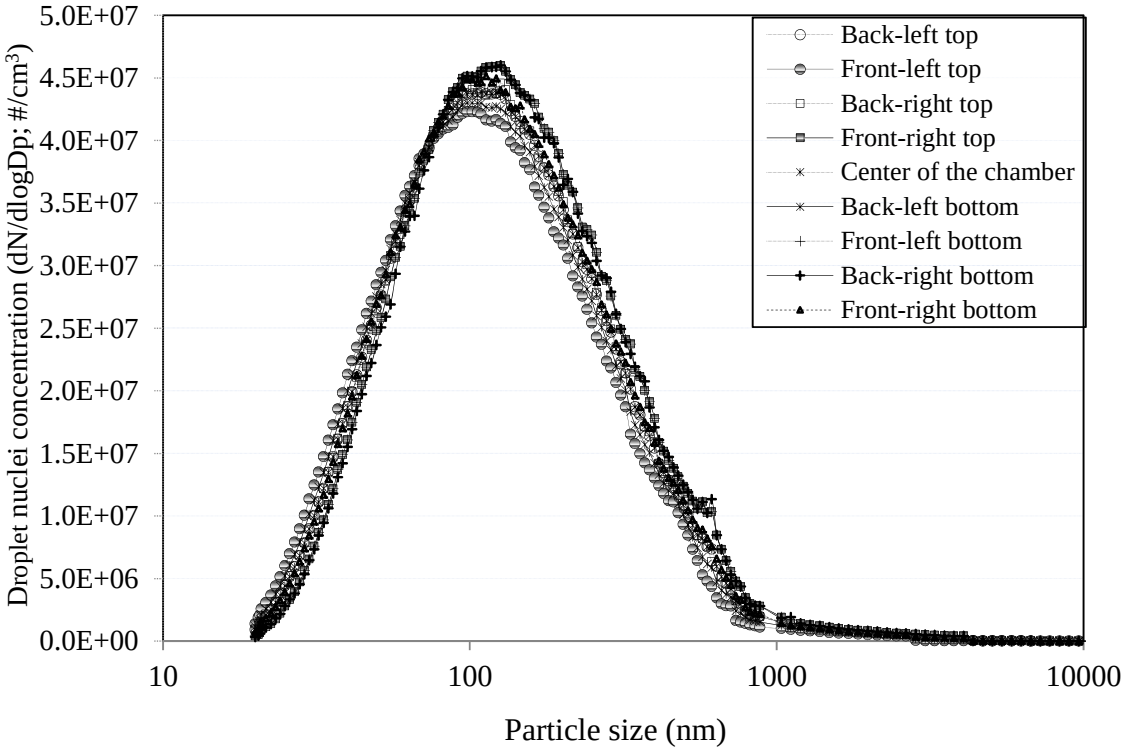

Fig. 5. Average size distribution of the aerosol from the droplet nuclei experiments at different chamber locations measured using the APS and SMPS (combined).

Viable MS2 extracted from the PPE

Using the 271B medium and the vortex mixer, extraction efficiencies > 99% were found for all types of filter media for MS2 delivered via a spiking procedure. The impact of nebulizer and VOAG on viability of the virus during particle generation was investigated by comparing the viable MS2 recovered from the Willson P100 FFR samples to those from a suspension solution. The theoretical concentration on the Willson P100 samples after a 22-min loading period was 2.18×10^5 PFU/cm^2. The average experimental concentration in the Willson P100 samples (n = 3) after a 22-min loading period was approximately 1.99×10^5 PFU/cm^2, which is very close (91.3%) to the theoretical value. As a control experiment, the survival of MS2 virus on the P100 FFRs at different storage times of 1, 7, 14, and 22 min, was found to be 1.70×10^4, 1.67×10^4 , 1.71×10^4, 1.72×10^4 PFU/cm^2, respectively. These results show that MS2 virus viability will not decrease during a 22-min loading experiment.

Uniform deposition was determined by assessing the viability of MS2 extracted from the contaminated PPE at different locations. The survival of MS2 droplet nuclei on the Gerson N95 FFRs was found to be 1.14×10^5, 1.05×10^5, 1.11×10^5, 1.10×10^5, and 1.09×10^5 PFU/cm^2 for the top, center, bottom, left, and right areas of FFR samples, respectively, while survival of MS2 droplets was found to be 1.19×10^5, 1.14×10^5, 1.11×10^5, 1.16×10^5, and 1.15×10^5 PFU/cm^2 for the top, center, bottom, left, and right areas of FFR samples, respectively. In general, all CVs for L-T-L were found to be ≤ 7.88% (Table II), which is lower than the target criterion (CV ≤ 20%). Measurements of viable MS2 droplets and droplet nuclei trapped on the three different FFRs and SMs were also determined (Table III). All CVs for S-T-S were found to be ≤ 6.01% (Table II), which is lower than the target criterion (CV ≤ 40%). Table IV displays the results of MS2 trapped within each FFR layer.

TABLE II. Location to Location and Sample to Sample Variability

PPE type	Particle type	Mean MS2 recovered (PFU/cm^2)[a]	CV for L-T-L (%)[b]	CV for S-T-S (%)[b]
Willson P100 P1130 M/L	Droplets (90% RH; 25 ºC)	$1.99 \times 10^5 \pm 2.0 \times 10^3$	2.95 ± 1.32	2.70 ± 0.78
	Droplet nuclei (35% RH; 23 ºC)	$1.90 \times 10^5 \pm 1.53 \times 10^3$	2.69 ± 2.16	2.10 ± 1.68
North N95	Droplets	$1.33 \times 10^5 \pm 2.08 \times 10^3$	3.83 ± 1.03	3.90 ± 1.22
	Droplet nuclei	$1.28 \times 10^5 \pm 2.1 \times 10^3$	3.92 ± 0.86	4.11 ± 1.13
Gerson N95	Droplets	$1.15 \times 10^5 \pm 1.53 \times 10^3$	4.67 ± 0.36	3.34 ± 1.78
	Droplet nuclei	$1.13 \times 10^5 \pm 2.0 \times 10^3$	4.79 ± 0.48	4.44 ± 0.90
Surgical mask 3M 1800	Droplets	$2.23 \times 10^4 \pm 5.03 \times 10^2$	7.88 ± 1.93	5.61 ± 1.32
	Droplet nuclei	$2.15 \times 10^4 \pm 5.13 \times 10^2$	7.21 ± 2.26	6.01 ± 2.17

[a] Mean MS2 ± σ (n = 3); [b] Mean CV ± σ (n = 3)

TABLE III. PPE Contamination Levels and Filter Efficiency

PPE Type	Particle type	MS2 recovered from PPE being tested (PFU/cm^2)[a]	Mean MS2 recovered from 1st downstream collection filter (PFU/cm^2; surface area = 5.07 cm^2)[a]	MS2 recovered from the 2nd downstream collection filter	FE[e] (%)
Willson P100 P1130 M/L (surface area[b] = 148 cm^2)	Droplets	$1.99x10^5 \pm 2.0x10^3$ (48717 PFU/cm^2/L)	$3.20x10^3 \pm 2.5x10^2$ (27 PFU/cm^2/L)	n.d.[c]	99.94
	Droplet nuclei	$1.90x10^5 \pm 4.7x10^3$ (46867 PFU/cm^2/L)	$4.89x10^3 \pm 2.65x10^2$ (41 PFU/cm^2/L)	n.d.[c]	99.91
North N95 (surface area[b] = 148 cm^2)	Droplets	$1.33x10^5 \pm 2.08x10^3$ (32716 PFU/cm^2/L)	$7.3x10^4 \pm 1.53x10^2$ (607 PFU/cm^2/L)	n.d.[c]	98.18
	Droplet nuclei	$1.28x10^5 \pm 2.1x10^3$ (31503 PFU/cm^2/L)	$8.48x10^4 \pm 3.06x10^2$ (719 PFU/cm^2/L)	n.d.[c]	97.77
Gerson N95 (surface area[b] = 148 cm^2)	Droplets	$1.15x10^5 \pm 1.53x10^3$ (28367 PFU/cm^2/L)	$8.87x10^4 \pm 3.1x10^2$ (750 PFU/cm^2/L)	n.d.[c]	97.42
	Droplet nuclei	$1.13x10^5 \pm 2.0x10^3$ (27778 PFU/cm^2/L)	$9.16\ x10^4 \pm 2.52x10^3$ (986 FU/cm^2/L)	n.d.[c]	96.57
Surgical mask 3M 1800 (surface area[b] = 148 cm^2)	Droplets	$2.23x10^4 \pm 5.03x10^3$ (5494 PFU/cm^2/L)	$1.58x10^5 \pm 4.51x10^3$ (1337 PFU/cm^2/L)	BMDL[d]	80.43
	Droplet nuclei	$2.15x10^4 \pm 5.13x10^3$ (5309 PFU/cm^2/L)	$1.7x10^5 \pm 4.58x10^3$ (1438 PFU/cm^2/L)	BMDL[d]	78.69

[a] Mean MS2 ± σ (n = 3); [b] surface area: FFR/surgical mask exposed area, excluding the FFR/surgical mask area that was sealed by silicone sealant to the face of the head form; [c]n.d.: No detectable MS2 survival was observed; [d]BMDL: Below the minimum detection limit of MS2 survival was observed; [e] calculated using equation 2.

TABLE IV. Viable MS2 Trapped Within Each FFR Layer

N95 FFR type	Particle type	Layer (material)	Average MS2 per cm^2 (PFU/cm^2)[a]	Percent
North N95	Droplets	Layer 1: Outer layer (hydrophobic)	$1.16x10^5 \pm 9.3x10^3$	59%
		Layer 2: Middle-a (hydrophilic + hydrophobic)	$3.7x10^4 \pm 4.0x10^3$	19%
		Layer 3: Middle-b (hydrophobic)	$4.2x10^4 \pm 5.5x10^3$	21%
		Layer 4: Inner layer (hydrophilic)	$2x10^3 \pm 3.9x10^2$	1%
	Droplet nuclei	Layer 1: Outer layer (hydrophobic)	$1.04x10^5 \pm 5.7x10^3$	56%
		Layer 2: Middle-a (hydrophilic + hydrophobic)	$3.4x10^4 \pm 4.6x10^3$	18%
		Layer 3: Middle-b (hydrophobic)	$4.2x10^4 \pm 6.2x10^3$	23%
		Layer 4: Inner layer (hydrophilic)	$6.0x10^3 \pm 2.5x10^2$	3%
Gerson N95	Droplets	Outer layer (hydrophilic)	$6.4x10^4 \pm 3.0x10^3$	40%
		Middle layer (hydrophobic)	$9.4x10^4 \pm 4.6x10^3$	59%
		Inner layer (hydrophilic)	$1.2x10^3 \pm 2.9x10^2$	1%
	Droplet nuclei	Outer layer (hydrophilic)	$6.1x10^4 \pm 6.7x10^3$	37%
		Middle layer (hydrophobic)	$1.0x10^5 \pm 3.6x10^3$	61%
		Inner layer (hydrophilic)	$3.2x10^3 \pm 2.6x10^2$	2%

[a] Average MS2 per $cm^2 \pm \sigma$ (n = 3)

Filtration efficiency for viable MS2 aerosol

A statistical summary of the PPE FE for viable MS2 is shown in Table III. During these experiments, the breathing machine reported airflow resistance resulting from the PPE seal to the headform. The mean breathing airway resistance was found to be 6.4 ± 0.1, 7.0 ± 0.06, 7.2 ± 0.06, and 7.9 ± 0.1 cmH_2O for the surgical mask, Gerson N95 FFR, North N95 FFR, and P100 FFR, respectively. As expected, average FE results (Table III) were highest for the P100 FFRs (99.91–99.94%), followed by the N95 FFRs (96.57–98.18%) and surgical masks (78.69–80.43%).

DISCUSSION

In this study, two experimental scenarios were evaluated to demonstrate the broad applicability of BARTS-II. The sizes and types of VCPs generated from this test system can be tailored toward the needs of an experiment by adjusting the temperature and RH inside the chamber, by varying the composition of the aerosol media (data not shown), modifying nebulizer parameters, or by changing the frequency or orifice size of the VOAG. In this study, we chose to operate the VOAG and the nebulizer simultaneously to create a polydispersed distribution of VCPs. Previous studies have used the nebulizer without a VOAG to generate a polydispersed distribution of submicron VCPs for respirator decontamination and reuse studies (Fisher, 2009; Vo, 2009; Heimbuch, 2011). At 23 ºC and 35% RH, VCPs generated from the nebulizer and VOAG rapidly evaporated to form viral droplet nuclei, ranging in size from 0.2 to 4.0 µm. Because the majority (>99%) of the viral droplet nuclei were ≤ 10 µm, these viral droplet nuclei may simulate those that contribute to aerosol transmission via inhalation. VCPs in this size range are consistent with experimental results. For example, 82% of the droplet nuclei measured from coughs of human subjects at 35% RH were between 0.74 – 2.12 µm (Yang, 2007). Using a cyclone sampler, Lindsley et al. (2010) found that 42% of the detectable influenza A collected from the air of a medical clinic was in particles ≤ 4.1 µm and 9% of the respiratory syncytial virus was ≤ 4.1 µm, while Yang (Yang, 2011) found that 64% of airborne influenza containing particles were < 2.5 µm.

The second experimental scenario presumably generates a “wet” VCP (i.e., droplet). Although no attempt was made to measure “wetness” directly, the assumption that some of the particles had not undergone complete evaporation was based upon the slightly larger size range, high RH (90%) in the chamber, and lack of dilution air for the nebulizer. The ability to generate a “wet” VCP is of interest to researchers. For example, one recent study from our laboratory (Fisher, 2012) found that reaerosolization of MS2 from an FFR contaminated via a direct-spray method (i.e., presumably wet) were different than from FFR contaminated with droplet nuclei. In this study, the size range for the droplet VCPs was 0.54-100 µm. The majority of the VCPs were centered in the range of 0.73-18.5 µm (with 83% < 10 µm), which span both the respirable and inspirable size ranges. Others have experimentally found polydispersed ranges of droplets in this size range. For example, using an APS, one study found human coughed droplets between 0.62–15.9 µm (Yang, 2007).

In general, the size distribution of the VCPs from the aerosol generators is controlled by the properties of the nebulizer and VOAG, particle generator liquid media, and dilution air, not by the physical size of the viruses themselves (Hogan et al., 2005). The environmental conditions that can affect the VCP size in the exposure chamber, such as RH and temperature, were relatively uniform throughout the chamber. Comparison of the results at each experimental condition indicated that the new test system not only generated a designated particle size, but also generated similar particle counts at different chamber locations.

The high recovery rates (91.3% of theoretical value) of MS2 from the contaminated Willson P100 FFR suggest that the nebulizer and VOAG did not cause significant damage to the MS2 during particle generation. These data also suggest that little particle loss was observed due to deposition on the walls of the chamber. Furthermore, the Willson P100 FFR yielded a high efficiency for capturing VCPs and recovering MS2 virus from the extraction process. Others have also observed good recovery rates using MS2 for contamination of PPE. For example, Woo et al. (2010) reported a high MS2 recovery rate when investigating the effect of an ultrasonic nebulizer generator during particle generation. Their results show that the experimental concentration (3.2×10^5 PFU/mL) of collected MS2 in the BioSampler was similar to the theoretical value (3×10^5 PFU/mL).

Contamination (e.g., deposition of the virus on the surface and trapped within the fiber webs of the non-woven filter layers) was found to be relatively uniform across the FFR. Comparison of these results at each experimental condition indicated that although the flexible nature of the FFRs resulted in a

shape that was not completely symmetric, the total amount of VCP at different FFR locations (top, center, bottom, left, and right areas) in BARTS-II was very similar. These results show that the new test system delivered a uniform concentration for both viral droplets and droplet nuclei onto different FFR locations. All CVs for uniform deposition, S-T-S and L-T-L, met the quality requirements for loading of ASTM E2720 and E2721. Interestingly, a majority (> 97%) of the MS2 was found on the outer and middle layers (Table IV).

Average FE results (Table III) were highest for the P100 FFRs (99.91–99.94%), followed by the N95 FFRs (96.57–98.18%) and surgical masks (78.69–80.43%). This trend is not surprising as NIOSH certified particulate respirators are tested for filtration efficiency using a near "worst-case" set of test conditions, while surgical masks are not (Rengasamy et al, 2009). Despite significant methodology differences, the FE trends found here are in agreement with those obtained by others. Using a wide-range particle spectrometer, one study found that the average penetration of particles from the nebulizer at an inhalation flow rate of 30 L/min were less than 3% and 4% for the two N95 respirators tested and less than 15% and 80% for the two SM models tested (Balazy et al., 2006).

Based on these promising results, BARTS-II is expected to find utility in updating standards and test methods for comparing PPE decontamination methods and in future research, such as loading surfaces to study fomite transmission and evaluating the impact of relative humidity and air temperature on survival of viruses and bacteria on PPE and environmental surfaces. Future studies will include tests designed to target larger particle size ranges and experiments to verify the "wetness" of the generated droplets. The simple methodology used here to obtain FE requires further validation. While the focus of this study was PPE using a biosafety level (BSL)-I virus, the system was also designed with a secondary containment to be possibly used with BSL-II microorganisms.

CONCLUSIONS

Overall, the results show that BARTS-II was capable of (1) producing uniformly deposited polydispersed distributions of VCPs onto PPE and (2) measuring the FE of PPE. Two aerosol challenges were evaluated by varying the aerosol generator operating parameters and the humidity in the test chamber. The amount of viable MS2 deposited on PPE met ASTM E2720-10 and E2721-10 quality requirements for loading, with 97% of the virus on the outer and middle layers of the FFR. Average FE was highest for P100 FFRs, followed by N95 FFRs and surgical masks. Based on these promising results, BARTS-II could find utility in updating standards and test methods for comparing PPE decontamination methods and for studying the role of PPE and surfaces in fomite transmission.

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Disclaimer

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