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VIRAL PENETRATION OF HIGH EFFICIENCY PARTICULATE AIR (HEPA) FILTERS

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14. ABSTRACT

High Efficiency Particulate Air (HEPA) filters are the primary technology used for particulate removal in individual and collective protection applications. HEPA filters are commonly thought to be impenetrable, but in fact they are only 99.97% efficient at collecting the most-penetrating particle (~0.3 micrometer). While this is an impressive collection efficiency, HEPA filters may not provide adequate protection for all threats: viruses are submicron in size and have small minimum infections doses (MID_{50}). Thus, an appropriate viral challenge may yield penetration that will lead to infection of personnel. However, the overall particle size (agglomerated viruses and/or viruses attached to inert carriers) will determine the capture efficiency of the HEPA filter. Aerosolized viruses are commonly thought to exist as agglomerates, which would increase the particle size and consequently increase their capture efficiency. However, many of the threat agent viruses can be highly agglomerated and still exist as submicron particles. We have demonstrated that MS2 coli phage aerosols can penetrate Carbon HEPA Aerosol Canisters (CHAC). At a face velocity of 2 cm/sec, a nebulized challenge of ~10^5 viable plaque forming units (PFU) per liter of air results in penetration of ~1 -2 viable PFU per liter of air. We are currently investigating the particle size distribution of the MS2 coli phage aerosol to determine if the challenge is tactically relevant. Preliminary results indicate that 200-300-nm particles account for ~7.5% of the total number of particles. Our aim is to characterize multiple aerosol conditions and measure the effects on viable penetration. This study will expand our knowledge of the tactical threat posed by viral aerosols to HEPA filter systems.

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Viral Penetration of HEPA Filters

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Abstract

High-efficiency particulate air (HEPA) filters are the primary technology used for particle removal in individual and collective protection applications. HEPA filters are commonly thought to be impenetrable, but in fact they are only 99.97% efficient at collecting the most-penetrating particle (~ 0.3 micrometer). While this is impressive collection efficiency, HEPA filters may not provide adequate protection for all threats: viruses are submicron in size and have small median infectious doses (MID_{50}). Thus, an appropriate viral challenge may yield penetration that will lead to infection of personnel. The overall particle size (agglomerated viruses and/or viruses attached to inert carriers) will determine the capture efficiency of the HEPA filter. Aerosolized viruses are commonly thought to exist as agglomerates, which would increase the particle size and consequently increase their capture efficiency. However, many of the threat agent viruses can be highly agglomerated and still exist as submicron particles. We have demonstrated that MS2 coli phage aerosols can penetrate carbon–HEPA aerosol canisters (CHACs). At a face velocity of 2 cm/sec a nebulized challenge of ~10^5 viable plaque-forming units

(PFU) per liter of air results in penetration of ~1–2 viable PFU per liter of air. We are currently investigating the particle size distribution of the MS2 coli phage aerosol to determine if the challenge is tactically relevant. Preliminary results indicate that 200–300 nm particles account for ~7.5% of the total number of particles. Our aim is to characterize multiple aerosol conditions and measure the effects on viable penetration. This study will expand our knowledge of the tactical threat posed by viral aerosols to HEPA filter systems.

Introduction

Biological Warfare/Terrorism is defined as actual or threatened deployment of biological agents to produce casualties or disease in man or animals and damage to plants or material. It is actually much farther reaching than that because contamination of infrastructure, which does directly affect individuals, is a concern due to the extensive and costly clean up required. The potential of biological weapons was demonstrated early in world history (Hawley 2001) starting in the 14th century when plague-infected carcasses were catapulted into enemy cities in an effort to spread the disease. Also, during the French and Indian war in 1754–1767, British soldiers provided American Indians with smallpox- contaminated blankets and handkerchiefs. These events predate Louis Pasteur's discovery that infectious diseases are caused by microorganisms, and clearly root biological agents as man's first attempt at creating a Weapon of Mass Destruction (WMD). Once microorganisms were linked to human disease, it did not take long for purified microbes to be used as weapons. It is well documented that many

countries, including the United States, had extensive bioweapons programs (Gronvall 2005, Frischknecht 2003). Perhaps the most feared was that of the Soviet Union. Human history is littered with many examples of microbes being deployed as acts of war and terrorism, the most recent documented example being the attack on the Hart Building in 2001. This single act of bioterrorism clearly demonstrated the potential threat that biological agents pose as a weapon of terror.

Biological agents are classified into four unique categories: vegetative bacterial cells, spores, viruses, and toxins; viruses are the primary concern in this report. Although the viral warfare agents are diverse and cause a variety of diseases, their physical properties are similar (Woods 2005): all contain a nucleic acid core surrounded by a protein coat; most also contain a lipid membrane, and are termed enveloped. Viruses are submicron particles, ranging in size from ~25–400 nm (Hogan 2005, Kowalski 1999) and the median infectious dose (MID_{50}) for all the threat agent viruses is very low. While absolute figures are not available, most believe that the MID_{50}s are less than ten virions (Woods 2005). The combination of small size and low infectious doses raises concern that high-efficiency particulate air (HEPA) filters may not adequately protect individuals from viral WMD.

HEPA filters are commonly used in individual and collective protection applications and are very efficient at removing particulate matter from the air. They are rated to be 99.97% efficient at collecting the nominal most-penetrating particle (0.3 μm) (Lee 1980). Although this collection efficiency is impressive, it is not absolute; depending on

conditions, 0.03% of matter at the most penetrating size does penetrate the HEPA filter. For most applications the HEPA is adequate, but tolerance for viral penetration is very low, and thus only a few penetrating virions may be enough to cause disease. For viruses to be efficient at penetrating HEPA filters they must remain as submicron particles. Most agree that viruses will not occur as singlets when dispersed in an aerosol; rather, they will agglomerate or attach to inert materials that will increase the particle sizes (Stetzenbach 1992). It is important to note, however, that many of the threat agent viruses (*e.g.*, SARS, EEV) can be significantly agglomerated and still fall into the most-penetrating range. Most of the research on bioaerosols has focused on naturally occurring biological aerosols. The research has demonstrated that a majority of particles in biological aerosols are greater than 1μm in size (Stetzenbach 1992), and thus would not be a threat to penetrate HEPA filters. It should be noted that the technology used in these studies is not able to effectively measure bioparticles smaller than 500 nm. Therefore, the abundance of particles that would be most efficient at penetrating HEPA filters was not properly quantified. Studies of naturally occurring particulate aerosols (non-biological) demonstrate that nanometer-size particles are actually abundant (Biswas and Wu 2005).

Weaponized viruses are clearly different from naturally occurring biological aerosols and the particle size for viral weapons is not clearly defined. From a weapons standpoint, it would be advantageous to create smaller particles, because they would remain aerosolized longer. But in addition to creating small particles one must preserve the viability of the viruses. The methods used to produce and protect viruses from environmental stress may dictate creating larger particles. It is unclear if weaponized

viruses have been created that are submicron in size. This uncertainty has fueled speculation that viruses may indeed be a threat to penetrate HEPA filters.

The study of viral penetration of HEPA filters dates back to the development of HEPA filters by the Department of Energy (DOE) in the 1950s (Mack, 1957). Since that time more than 20 published studies have used a variety of experimental techniques to quantify viable penetration of HEPA filters. A comprehensive review of these studies edited by Wander is due to be published in 2010. Six studies (Decker 1963, Harstad 1967, 1969, Roelants 1968, Thorne 1960, and Washam 1966) were published in the 1960s; all were chamber tests aimed at determining the viable filtration efficiency of the media and/or devices. The most elegant of these studies were carried out by Harstad, who observed that the principal route of penetration is filter defects (pinhole leaks, media breaks due to pleating, etc.) and not through the medium itself. The next 30 years produced only eight research articles, six chamber tests (Bolton 1976, Dryden 1980, Eng 1996, Leenders 1984, Rapp1992, and Vandenbroucke–Grauls1995), and two studies that used an animal model (Burmester 1972, Hopkins1971) to assay the protection provided by HEPA filters. The turn of the 21st century saw a renaissance of interest in research on viral penetration of HEPA media—a total of seven articles were published in seven years. Research on active processes for air purification (reactive/antimicrobial media, heat, energetic light, etc.) that kill microbes rather than just capture them was the main driver for these studies (Heimbuch 2004, Lee 2008, Ratnesar 2008, and RTI 2006). Dee *et al* (2005, 2006$_a$, 2006$_b$) also performed three studies using a swine model to determine the effectiveness of HEPA filters

The review of all research studies dating back to Mack's report in 1957 reveals a common theme: HEPA filters provide HEPA-level performance (> 99.97% efficiency), which was duly noted by the authors. Many of these authors could also have concluded that their studies demonstrated that viable viruses penetrate HEPA filters at levels that may cause disease. The purpose of this report is to reanalyze the issues surrounding viral penetration of HEPA filters, and to shed new light on the potential for penetration. Furthermore, the protection afforded by the carbon HEPA aerosol canister (CHAC) is also specifically addressed. We demonstrated (Heimbuch 2004, Figure 1) in previous studies that viable MS2 coli phage can penetrate CHACs. However, these studies did not discriminate between penetration due to viruses passing through the HEPA medium and due to viruses bypassing the medium through defects in the canisters. In this study, the viral simulant MS2 coli phage was used to challenge both flat-sheet HEPA material and CHACs. Both viable penetration and total penetration were measured. In addition, particle size distribution and filtration velocity were varied to measure what effect each had on total and viable penetration.

Materials and Methods

Microorganisms: MS2 coli phage (ATCC 15597-B1) stock solutions were prepared by infecting 100 mL of the *Escherichia coli* host (ATCC 15597) that was grown to mid-log phase in special MS2 medium (1% tryptone, 0.5% yeast extract, 1% sodium chloride, .01 *M* calcium chloride, 0.002% thiamine). The infected culture was incubated overnight @

37ºC/220 rpm. Lysozyme (Sigma, L6876) was added to a final concentration of 50 μg/mL and the flask was incubated for 30 minutes at 37ºC. Chloroform (0.4%) and EDTA (.02 *M*) were then added and the culture was incubated for an additional 30 minutes at 37°C. Cell debris was removed by centrifugation at 10,000 X *g*, then the supernatant was filtered thorough a 0.2-μm filter and stored at 4ºC. A single-layer plaque assay was performed according to standard procedures (EPA) to determine the MS2 titer, which typically is ~10^{11} plaque-forming units (PFU)/mL. For aerosol studies, the MS2 coli phage was diluted in either sterile distilled water or 0.5% tryptone to a concentration of ~10^{8} PFU/mL.

Aerosol Methods: The BioAerosol Test System (BATS, Figure 2) is a port-accessible aerosolization chamber communicating with a temperature/humidity-controlled mixing plenum and thence to a sampling plenum supplying a homogeneous aerosol to six sampling ports. Three six-jet Collison nebulizers (BGI Inc, Waltham, Mass.) deliver droplets at the source that are ~2 μm mass median diameter into the mixing plenum to create the bioaerosols. Air is drawn into a central vacuum line along a path from the sampling plenum through lines of PVC tubing (Excelon® RNT, US Plastics, Lima, Ohio). Each path runs through a test article and thence through one AGI-30 all-glass impinger (Chemglass, Vineland, N.J.) filled with 20 mL of 1X phosphate buffer saline/0.001% antifoam A (Sigma, A6457). The volume of air passing in each path is controlled by a rotameter (Blue–White 400, Huntington Beach, California, or PMR1-101346, Cole–Parmer, Vernon Hills, Illinois). At the end of the sampling path, the air exhausts through a conventional HEPA filter and the vacuum pump that drives the air

movement. Each sampling port is able to accommodate test articles as large as 6 inches (15 cm) in diameter.

The BATS was configured three separate ways depending on what was being tested (Figure 3). In each case, the total flow through each port of the BATS was set to 85 liters per minute (LPM). The environmental conditions for all tests were ~22°C and 50% relative humidity. For flat-sheet HEPA testing, a portion of the flow was split off the 85-LPM flow and directed through the HEPA material (Lydall; Manchester, Conn.; part number 4450HS) that was compression seated and glued into swatch holders (Figure 3). For CHAC tests the entire 85-LPM flow was drawn though the CHAC, but only 12.5 LPM was collected in the AGI-30 impinger (Figure 3). For each test a portion of the flow was directed through a model 3936 Scanning Mobility Particle Sizing Spectrometer (SMPS) (TSI Inc, Shoreview, Minn.) that was configured to analyze particles with a diameter of 10 nm – 415 nm. The sample flow through the SMPS was 0.6 LPM with a sheath flow rate of 6 LPM.

Viable enumeration of MS2 coli phage was achieved by performing a plaque assay on the collection fluid from each AGI-30 impinger. One mL of solution from each impinger was mixed with 1 mL of log-phase *E. coli* grown in special MS2 medium. This solution was then mixed with 9 mL of semi-solid medium (special MS2 medium + 1% agar) that had been incubated at 55°C. The solution was poured into sterile Petri dishes and allowed to solidify. The plates were incubated at 37ºC overnight, then plaques were counted. The total collected phage for each impinger was determined using the following formula:

$$\text{Total PFU} = \text{counted PFU} \times \text{dilution}^{-1} \times \text{impinger volume}$$

Experimental Plan: At each condition tested in this study, six samples were challenged with MS2 coli phage over two days of testing: three samples and one positive control were analyzed each day. After the filters were seated into the swatch holders they were initially leak checked by challenging with an aerosol of 100-μm beads for 5 minutes. After the leak test the BATS was loaded with MS2 coli phage and equilibrated for 15 minutes prior to starting the challenge. The challenge comprised four 15-minute intervals, in which new impingers were installed after each interval. The SMPS incrementally analyzed penetration for each of the four swatch holders (three filters and one positive control) for 12.5 minutes of each 15-minute challenge period.

Explanation of flow rates and face velocity: The coupon samples used for this study were all 4.7-cm diameter circles, resulting in a surface area of 17.34cm^2. The flow rate through each filter was 2 LPM, 4 LPM, 6 LPM, or 8 LPM. Face velocities were calculated using the following formula:

$$\text{Face velocity (cm/sec)} = \text{flow rate } (cm^3/sec) \div \text{surface area } (cm^2)$$

The resulting face velocities were numerically equal to the flow rate (*i.e.*, 2 LPM rate = 2 cm/sec face velocity, 4 LPM flow rate = 4 cm/sec face velocity, etc). For the CHAC the entire surface area of the pleated HEPA filter was taken into account when calculating the

face velocity. The CHACs used in this study contained 750 cm^2 of HEPA medium that was tested at a flow rate of 85 LPM. The resulting face velocity, using the above formula, was 2 cm/sec.

Results

Size distribution of MS2 aerosols in the BATS: The SMPS analysis of MS2 aerosols created in the BATS revealed that the number mode diameter was ~35 nm and the mass mode diameter was ~ 151 nm (Figure 4). Both are composed of distributions that span the entire data collection range of the SMPS. By number, the fraction of particles that fall into the most-penetrating range for HEPA filters (100–300 nm) was only 7.5%. The curve for the mass distribution is not complete, but if we assume the curve is symmetrical, a reflection around the midpoint indicates that only 94% of the curve is represented by the data. The correction reveals that the amount of mass in the 100–300 nm range is 58%. Both number distribution and mass distribution of particles have been used by researchers for determining filter efficiency, but it is unclear which is more appropriate. For this analysis, the mass distribution specifies a much more stringent challenge for HEPA filters than does the number distribution.

Particulate penetration of flat sheet HEPA filters: The SMPS analysis (number and mass distributions) of the MS2 aerosols confirmed that the particle distributions and overall challenge levels for each flow rate were similar (Figure 5). This indicates a high degree of repeatability in the experimental setup. Penetration of particles through the

HEPA filter increased as flow rate increased (Figure 5). This indicates the HEPA filter becomes less efficient with increasing flow rate, as expected in size regions in which diffusional capture mechanisms dominate. At the low challenge concentrations (beginning and end of curves) the penetration data disappeared into the background and thus were not meaningful. When particle penetration experiments are done for HEPA filters, the particle challenge concentration is orders of magnitude greater than what can be created for biological challenges. Thus the signal-to-noise ratio is much larger. Analysis of penetration efficiency demonstrates that the most-penetrating particle (MPP) at the higher velocities is ~ 135 nm (Figure 6). The lower flow rates have limited overall penetration and an MPP size can not be discriminated. The MPPs for HEPA filters are commonly believed to be 300 nm, but it is actually closer to 200 nm (Lee 1980). The smaller MPP observed in this study is likely due to the higher flow velocities used in this study.

Viable MS2 penetration of flat-sheet HEPA filters: The viable MS2 penetration data indicate that as the flow rate increases, penetration through the HEPA also increases (Figure 7); this is in perfect agreement with the SMPS data. The difference in viable penetration increased ~1 $\log_{10}$ order of magnitude as the flow rate doubled. The increase in average penetration between the 2-cm/sec and 4-cm/sec velocity was just shy of the 1 $\log_{10}$ mark; this may be attributed to the overall low number of plaques detected for the 2 cm/sec assay. Also, the addition of the 4-LPM purge may have added additional variability. The overall viable penetration values are lower than what is reported for the particulate data. The reason for this is unclear, but viable assays are complex in

comparison to the SMPS analysis. The SMPS measures all particles regardless of whether or not they are viable or even contain a virus. The viable assay measures only viable MS2 particles. The differences in penetration between the assays indicate that viable MS2 is not evenly distributed across the entire particle size distribution.

Particle penetration of CHACs: The penetration of particles through the CHAC tracked most closely with the HEPA penetration data at 2 cm/sec (Figure 5). This was expected because the test flow rate of 85 LPM through the CHAC provides a velocity of 2 cm/sec through the CHAC HEPA filter. Analysis of the filtration efficiency (Figure 6) demonstrates that penetration through the CHAC also follows the penetration observed for flat- sheet HEPA material at velocities of 2 cm/sec and 4 cm/sec. The overall penetration was very low and a determination of MPP size was not possible.

Viable MS2 penetration of CHACs: MS2 penetration of the CHAC canister was lower than through any of the flat-sheet HEPA materials tested (Figure 7 and Table 1). The penetration most closely resembled that at 2 cm/sec velocity through the HEPA, as was expected due to similar face velocities, but the total measured penetration was only 1/7 of that through the flat sheet HEPA medium. The decrease in penetration through the CHAC was likely due to the presence of the carbon bed. The carbon bed adds more surface area for the aerosol to travel through, which could mechanically trap the MS2 particles. However, the SMPS analysis demonstrated the particle collection efficiency of the CHAC was very similar to the collection efficiency of the HEPA at the same velocity (2 cm/sec) (Figure 6). Thus, other mechanisms must be responsible for the viable reduction.

One possibility is that the additive ASZM-TEDA (Antimony–Silver–Zinc–Molybdenum–Triethylenediamine) in the carbon bed is exerting a biocidal effect on the bacteriophage. ASZM-TEDA is added to the carbon to prevent microbial growth and it may have virucidal activity as well.

Particulate penetration of 0.5% tryptone nebulization solution: The addition of tryptone (0.5%) to the nebulization fluid significantly shifted the size distribution of particles to the right (Figure 8). The number mode diameter shifted to ~89 nm and the mass mode diameter shifted to ~300 nm; the percentage of particles, by number, that fell into the 100–300 nm size range also increased by 28.5%. The mass curve was not complete, and thus the fraction of particles in the 100–300 nm size range could not be definitively calculated. However, if we assume the curve to be symmetrical the mass present in the 100–300 nm size range is 43%, a decrease of 15% over what is observed for MS2 suspended in water. The overall numbers of particles generated by MS2 nebulized in 0.5% tryptone and MS2 nebulized in water were not significantly different. The reason for this is that the output of droplets from the Collison nebulizer is constant regardless of what is being nebulized, so the addition of tryptone to the nebulizer did not affect the rate of generation of particles but rather altered the composition of the droplets. The increase in dissolved solids in each droplet produced by the Collison thus dramatically increased the total mass, with the net result that the MS2 coli phage was significantly loaded with protein. Delivery of the extra mass caused the HEPA filters to load with tryptone and they become more efficient over time (Figure 9). Filter loading

was not observed for MS2 suspended in water, and penetration remained constant during our experiments.

Viable MS2 penetration of 0.5% tryptone nebulization solution: The addition of tryptone to the nebulizer did not positively or negatively influence the viability of MS2 coli phage (Figure 10): both conditions of delivery yielded approximately the same concentration of viable MS2, but the addition of tryptone caused a significant decrease in penetration of MS2 coli phage through the HEPA filter over the entire sampling times (Figure 10). The initial decrease in viable penetration (Figure 10) was likely caused by the shift in particles away from the most penetrating size (Table 2). The mass distribution showed a 15% decrease in particles in MPP size, but the number distribution showed an increase of 28.5% MPP size. It would appear that the mass distribution is more relevant than the number distribution for determining viable penetration by MS2. Viable MS2 penetration also decreased over time and tryptone loading of the HEPA filter was likely responsible. No pressure drop measurements were made, but an increase in pressure loss with time would have been expected.

Discussion

Data presented in this report conclusively demonstrate that viable viruses can penetrate HEPA filters. This should not be surprising given the fact that HEPA filters are rated to be only 99.97% efficient at collecting 0.3-μm particles. Hence, given a sufficient challenge, penetration is a mathematical expectation. The penetration is small relative to

the challenge, and for most particulate challenges this minimal penetration is not problematic. Viruses, however, pose a unique problem because very few virions are required to cause an infection (MID_{50} < 10 PFU). This problem is further exacerbated because viruses are very small (25–400 nm), so individual viruses, and aggregates of viruses fall into the MPP range of HEPA filters. The data in this report were gathered from carefully controlled laboratory experiments—such an approach was necessary to evaluate viable penetration efficiency of HEPA filters. The tactical relevance of these data is a more-challenging problem because no criteria are available to determine that the BATS challenge is—or is not—representative of a biological attack. To determine if viral penetration of HEPA filters is a potential concern, four characteristics of viral aerosols must be considered: 1) Filtration velocity (flow rate), 2) Virus concentration, 3) Duration of the biological attack, and 4) Particle size. Each of these characteristics (discussed below) will significantly impact viral penetration of HEPA filters, and ultimately determine that HEPA filters do or do not provide "complete protection" against respiratory infection by airborne viruses.

The concentration of viruses created during a biological attack is not known. The concentration will likely vary depending on distance from the distribution source. The measured concentration of viruses for this study was only 10^4–10^5 PFU per liter of air. These concentrations are not excessively high and are likely lower than what would be generated during a biological attack. The duration of time that this concentration can be maintained is also an important parameter, as it directly relates to time of exposure. While there is no clear answer to this question, we do know that the penetration data

observed in this study were approximately linear over time. Therefore we can predict that penetration occurs instantaneously. This may be surprising to some but HEPA filters are an "open system" that contains holes. The SMPS analysis of HEPA penetration, which was measured over the duration of the challenge, confirms that particle penetration occurs instantaneously during a challenge. These data indicate that, given an appropriate challenge, an infective dose of viruses could be delivered in a matter of seconds following a challenge.

Flow rate and ultrafine particle penetration are directly related. As flow rate increases, penetration near and below the MPP size will increase. HEPA filters are commonly rated for a face velocity of $\leq$ 3.5 cm/sec to maintain the 99.97% collection efficiency and maximum pressure drop ratings. (Liu 1994, VanOsdell 1990). Our study confirms this, demonstrating that the 4-cm/sec velocity is the cutoff for obtaining HEPA performance for particle penetration. Viable MS2 coli phage penetration also increases with flow rate, with a significant increase in penetration at the higher velocities. For individual protection applications, the National Institute for Occupational Safety and Health (NIOSH) recommends a testing flow rate at 85 LPM; that equates to a 2-cm/sec filtration velocity for CHACs. However, breathing is more complex than simply testing at a uniform flow rate. Cyclic breathing will obviously allow penetration only during inhalation, and the most penetration will occur during peak flow velocities. Anderson *et a*l (2006), demonstrated that maximum peak flows for average males range from 125 LPM to 254 LPM depending on work load (light to heavy). Peak flow was cyclic and

accounted for ~ ½ the total time tested. This indicates that an average male can inhale particles at velocities greater than the rated velocities for HEPA filters.

The particle size distribution for this study was very small and may not be representative of a viral weapon attack; only 7.5% of the particles by number fell into the most-penetrating range. In an effort to shift the particle distribution to the right, tryptone was added to the nebulization fluid. This generated more particles (by number) in the most-penetrating range (Figure 8, Table 2), but the net result was a decrease in viable penetration (Figure 10). The result is counterintuitive, but if one considers the mass data, which showed a decrease in particles in the MPP size range (Table 2), then a decrease in viable penetration would be expected. Furthermore, the addition of tryptone caused a decrease in the production of particles with diameters ranging from 10 nm–100 nm (Table 2). Diffusional capture, which becomes less efficient as velocity increases, is responsible for collecting particles in this size range. The comparison of aerosolization of MS2 in tryptone solution vs. water was done only at 8 cm/sec velocity; thus the efficiency of diffusional capture was reduced, resulting in more penetration for the water aerosolization, but not significantly impacting the tryptone aerosolization. These combined factors contributed to a 2-log decrease in penetration of viable MS2 virions. The viable penetration was further decreased over time, as a result of tryptone loading the HEPA filter and increasing the efficiency of the filter. The SMPS data clearly shows the time-based increase in filter efficiency for the tryptone aerosolization, but not for the water aerosolization (Figure 9).

The distribution of MS2 virions among inert particles is an important parameter that will affect viable penetration of HEPA filters. During nebulization, MS2 virions should be evenly distributed throughout the particle distribution regardless of the composition of the nebulization fluid. In practice nebulization is a harsh process that is known to kill microorganisms (McCullough 1998, Reponen 1997, Mainelis 2005). Viability of the microorganisms will also be reduced once the water has evaporated from the droplet. These factors may have contributed to the reduction of viable MS2 coli phage penetration of the HEPA, during the tryptone aerosolization (assuming that larger particles will be more likely to contain viable virions). Tryptone is reported to protect viruses from desiccation during aerosolization (Dubovi 1970), but our data indicate that aerosolization from tryptone solutions and from water delivered the same amount of viable MS2 coli phage (Figure 10). Therefore, one cannot assume that a proportionally greater number of viable MS2 virions are present in larger particles. Unfortunately technology is not available to determine real-time distribution of viable microorganisms within a particle or distribution of particles. Collection of MS2 in impingers, as was done for this study, can reveal only the viable MS2 virions per collection period, but does not provide information on particle size.

Summary

HEPA filters are designed to allow penetration of $\leq$ 0.03 % of challenging 0.3-μm particles. Viruses are simply particulate matter that will penetrate HEPA filters with the same efficiency as inert aerosols. This was clearly demonstrated in this study. What is not

clear is the relevance of this finding to biological attack scenarios involving weaponization of viruses. Biological aerosols are complex, and many factors must be considered. The data in this report both support and refute the scenarios required for viral penetration of HEPA filters. One of the key elements that is difficult to quantify is the term “weaponization.” Can viruses be prepared for tactical deployment so that they penetrate HEPA filters efficiently and still remain infectious? The answer to this question is not readily available, but the capability is not completely unlikely. A thorough examination of past biological weapons programs might provide some answers, but those data are hard to obtain and if available, still may not provide clear answers because historical bioweapon research appears to have assumed no respiratory protection. In the absence of those data, the certain way to know if HEPA filters provide adequate protection would be to create tactically relevant biological aerosols and determine their penetration efficiency through the HEPA filters. As a complicating factor, this type of research leads to a conundrum that many face in biological defense applications: the research is crucial to determine if a protection gap exists, but the research might also lead to conditions that could defeat the HEPA filter. This issue notwithstanding, basic research is needed to develop a better understanding of how viruses and other microbes behave in aerosols. In particular, the distribution of viruses, both viable and nonviable, among inert particles in aerosols is not well understood. Data generated from this type of research will help solve biological defense questions, but they will also further basic understanding about and control of the spread of infectious diseases.

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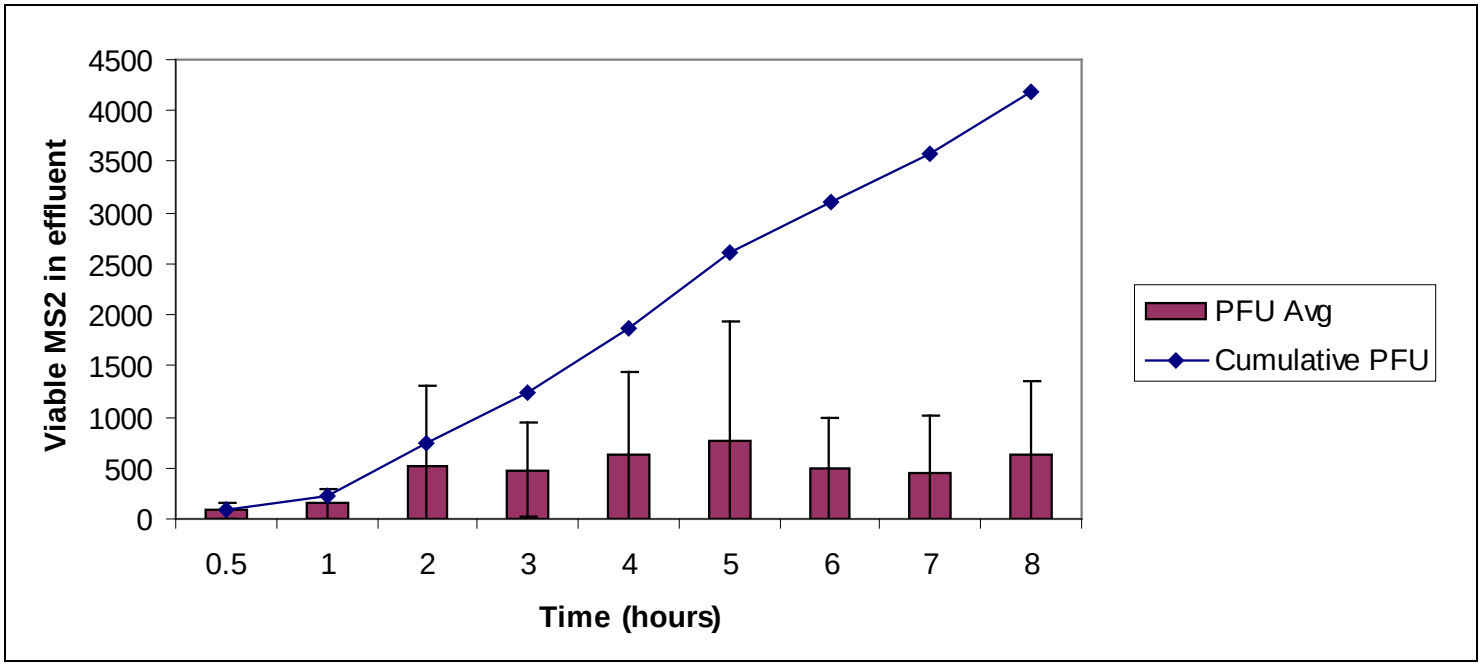

Figure 1: MS2 challenge (10^3 - 10^7 PFU/L of air at 85 LPM) of CHAC (n= 21) in BATS

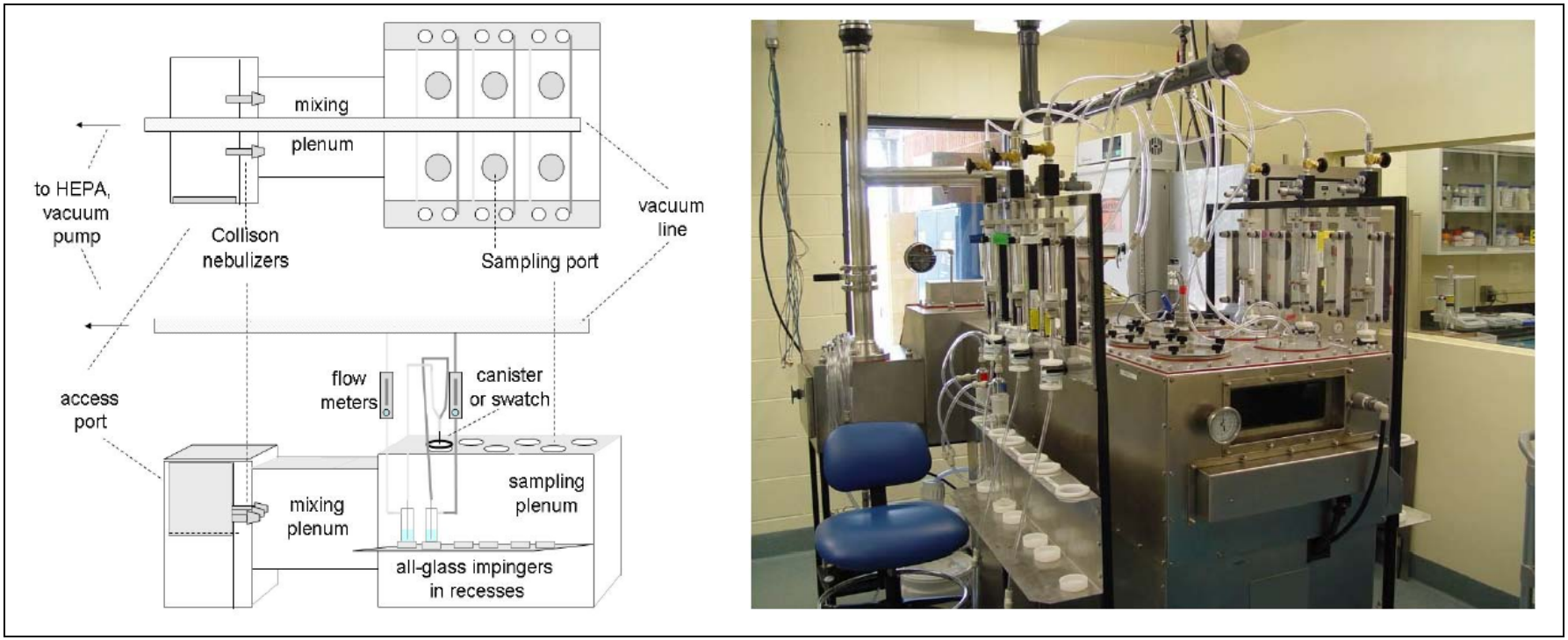

Figure 2: The BioAerosol Test System (BATS) is a Port-Accessible Aerosolization Chamber That is Capable of Safely Generating and Containing BSL-2 Biological Aerosols.

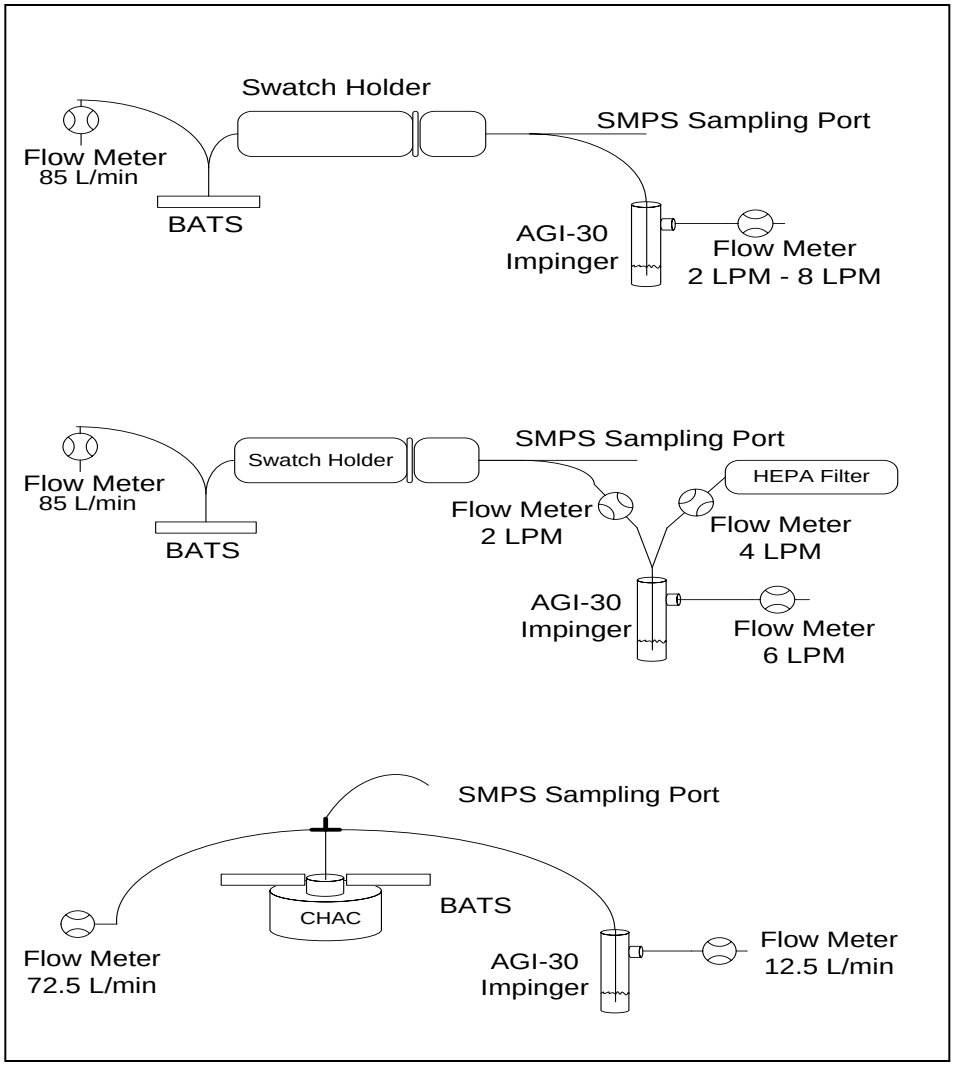

Figure 3: Three Test Configurations for Challenging Flat-sheet HEPA Material and CHACs with MS2 Coli Phage: The overall design allows for airflow downstream of the test article both to be analyzed by the SMPS and to be Collected in an all-glass impinger, allowing for assessment of viable penetration. 3a) The airflow through the BATS was 85 LPM and a split stream of either 2 LPM, 4 LPM, 6 LPM or 8 LPM was directed through the flat-sheet HEPA material. 3b) Purge air (4 LPM) was fed to the impinger to deliver an net 6 LPM to maintain collection efficiency (2 LPM through the HEPA filter plus 4 LPM purge). 3c) A CHAC was fixed to the BATS and the total airflow of 85 LPM was drawn through the canister.

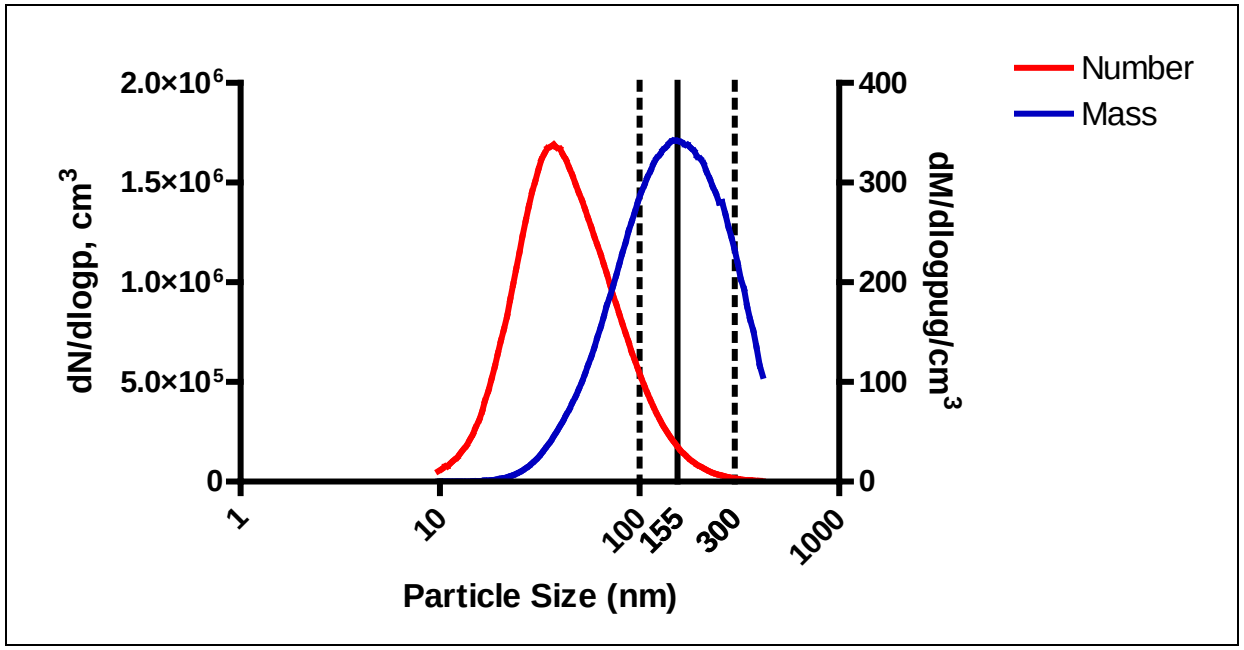

Figure 4: SMPS Analysis of MS2 Aerosolized in Water Using the BATS

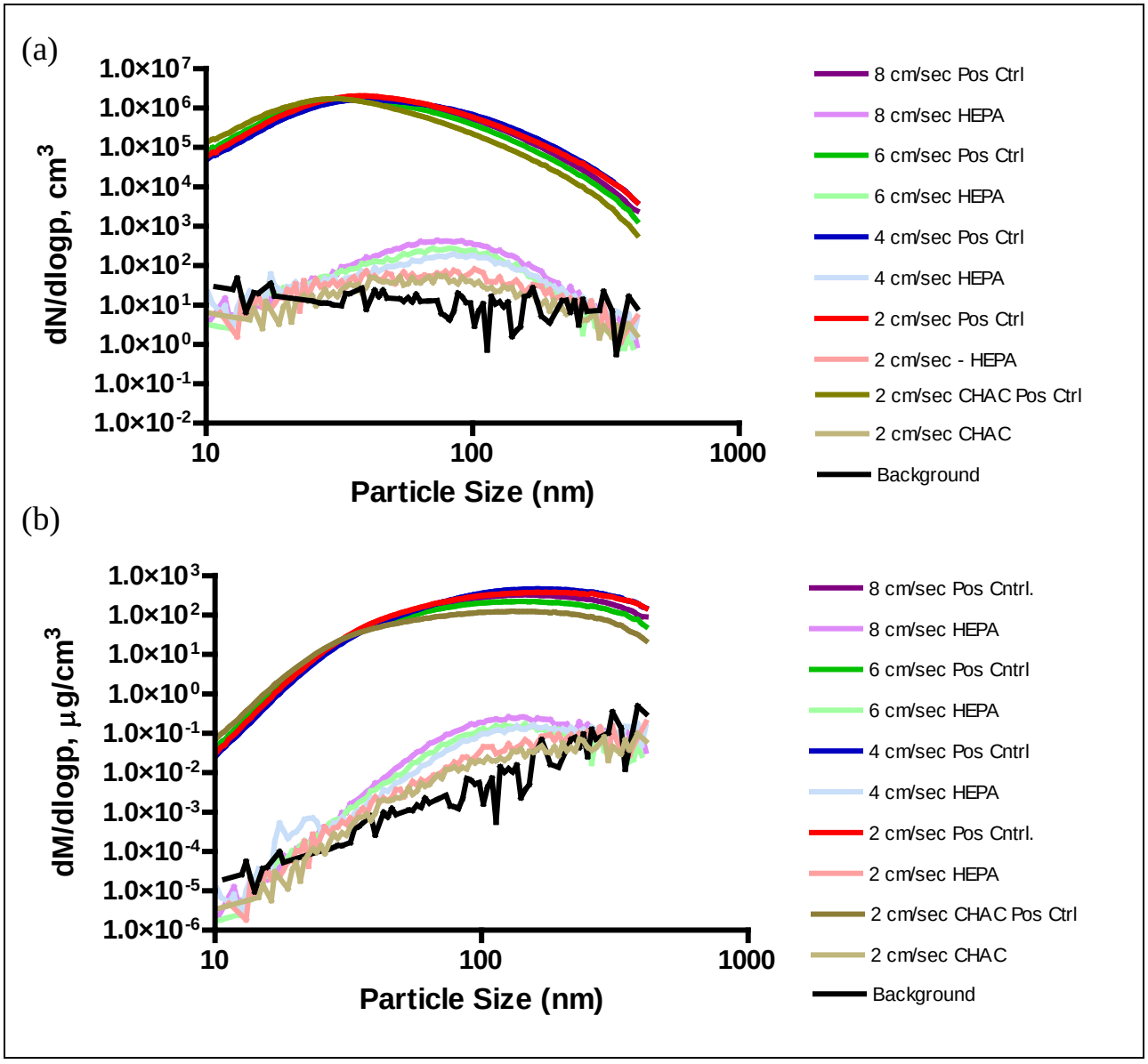

Figure 5:SMPS Analysis of MS2 Coli Phage Challenge of Flat-Sheet HEPA and CHAC [(a) Number , (b) Mass]

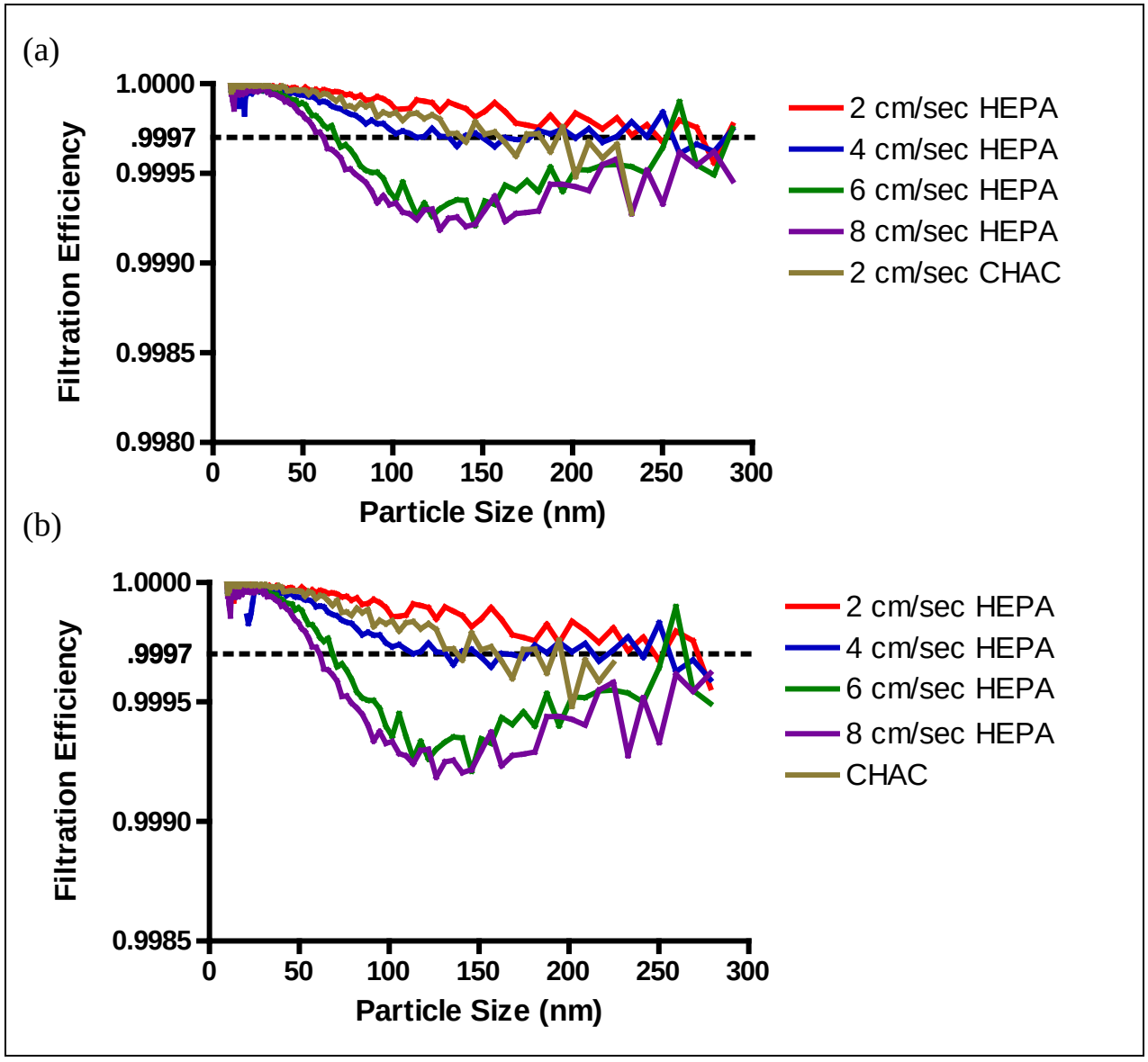

Figure 6: Filtration Efficiency of Flat-Sheet HEPA Challenged with MS2 Coli Phage [(a) Number , (b) Mass]

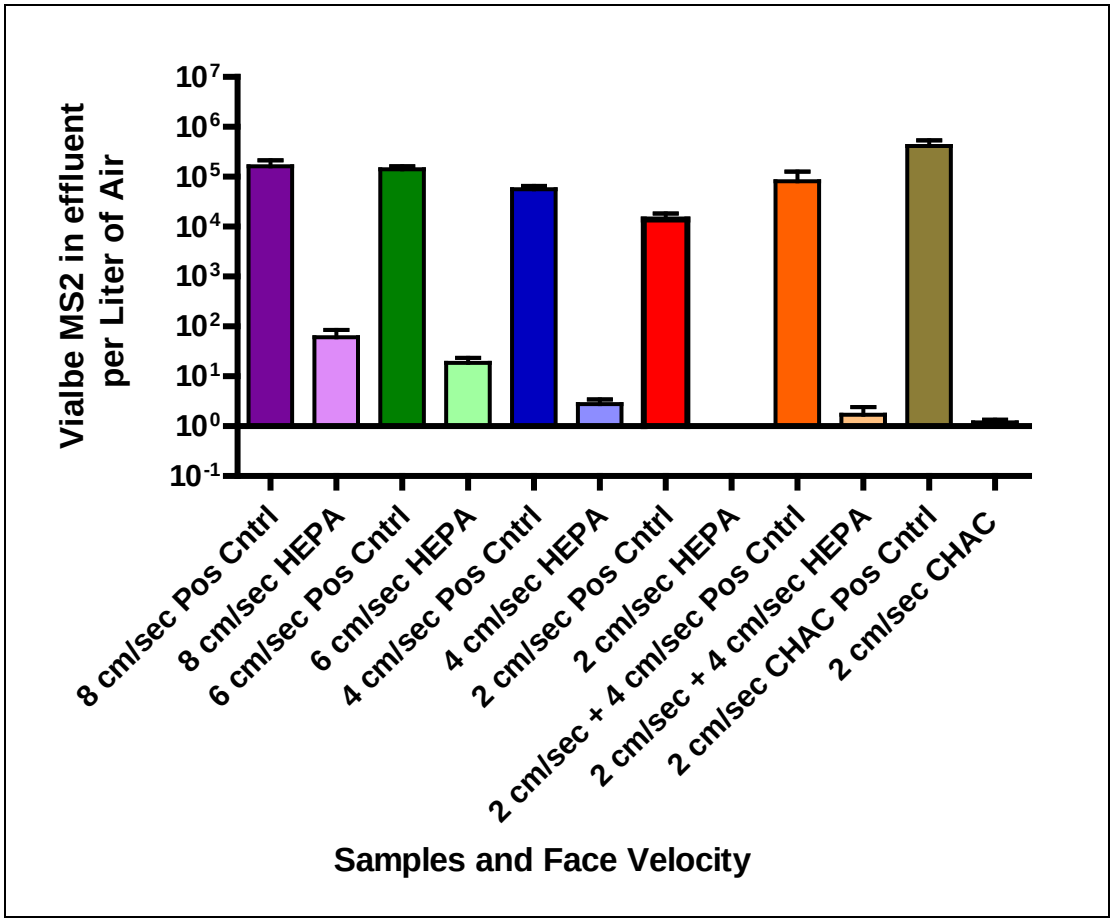

Figure 7: MS2 Challenge of Flat Sheet HEPA and CHAC—Viable Enumeration

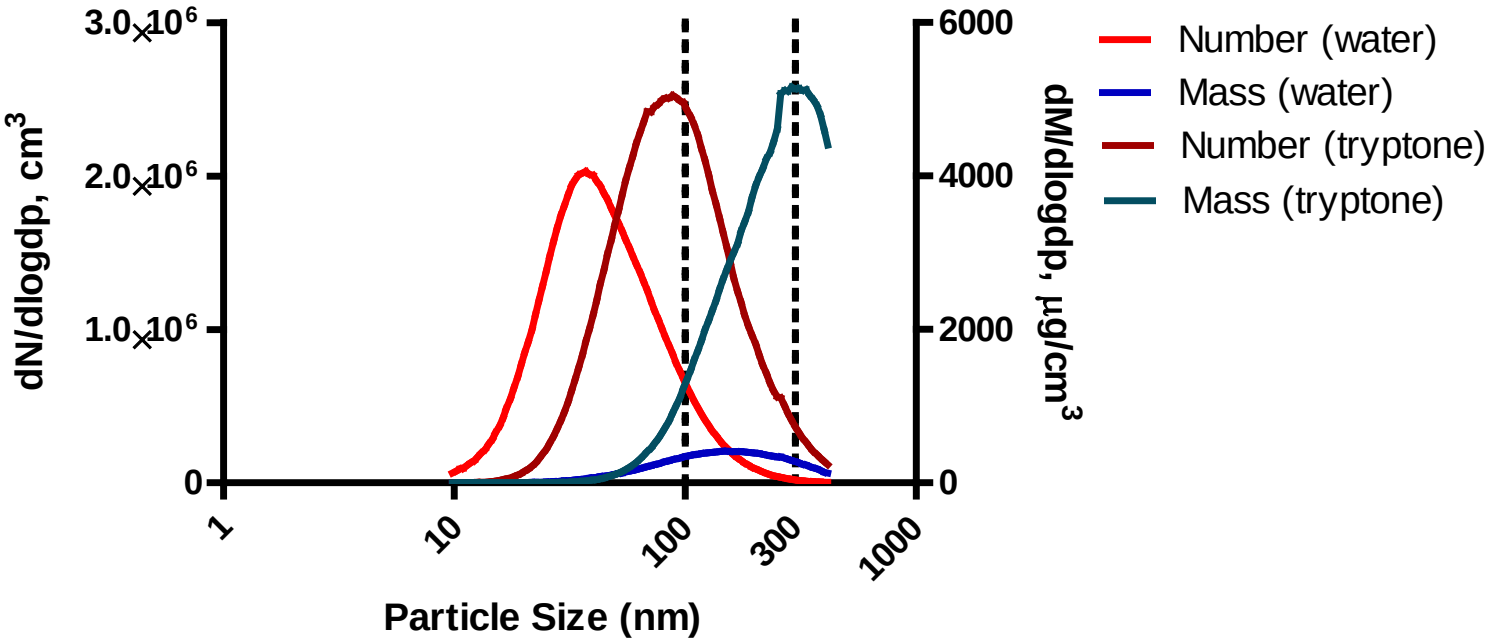

Figure 8: SMPS Analysis: Filtration Efficiency of Flat-Sheet HEPA Challenged with MS2 Aerosolized in 0.5% Tryptone and Water

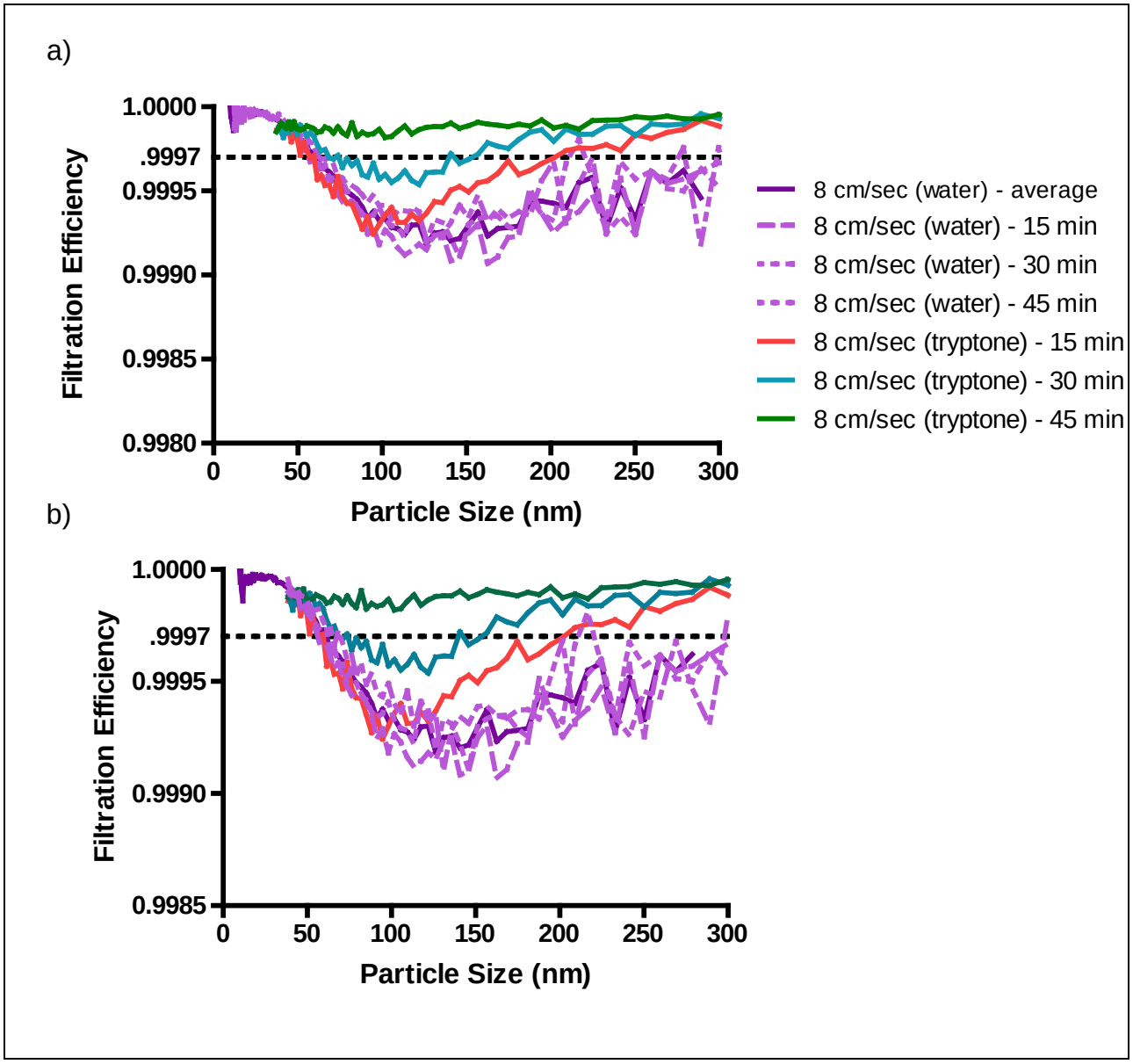

Figure 9: SMPS Analysis of Flat-Sheet HEPA Challenged with MS2 Aerosolized in 0.5% Tryptone and Water

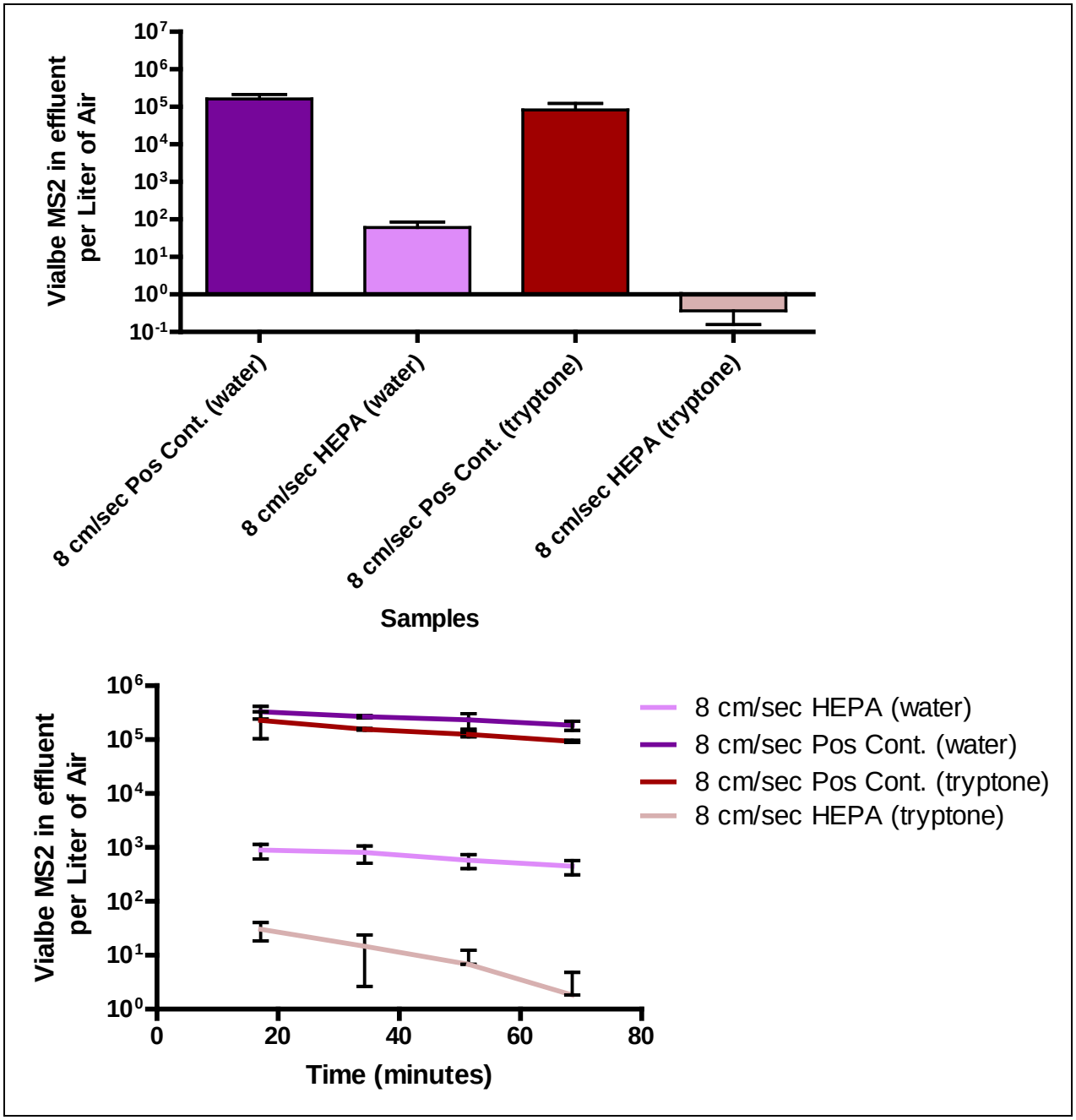

Figure 10: Viable Enumeration of Flat-Sheet HEPA Challenged with MS2 Aerosolized in 0.5% Tryptone and Water

Table 1: MS2 Challenge of Flat-Sheet HEPA and CHACs

Sample	Face Velocity	Collection Flow Rate	Average	Lower 95% CI	Upper 95% CI
Flat Sheet HEPA	2 cm/sec	2 LPM (+4 LPM into impinger)	99.9979%	99.9973%	99.9985%
Flat Sheet HEPA	4 cm/sec	4LPM	99.9951%	99.9941%	99.9961%
Flat Sheet HEPA	6 cm/sec	6 LPM	99.9888%	99.9871%	99.9905%
Flat Sheet HEPA	8 cm/sec	8LPM	99.9626%	99.9571%	99.9681%
CHAC	2 cm/sec	85 LPM	99.9997%	99.9996%	99.9999%

Table 2: Particle Size Distribution of MS2 Aerosolized in Water and 0.5% Tryptone

	Number Distribution		Mass Distribution*	
Particle Size Diameter	Water	0.5% Tryptone	Water	0.5% Tryptone
10 nm–100 nm	92%	62%	26%	5%
100 nm–300 nm	7.5%	36%	58%	43%
> 300 nm	0.1%	2%	15%	52%

*Data were corrected to account for the entire curve, which was not collected by the SMPS (see fig 8)