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**PREPRINT**

# **VIRAL PENETRATION OF HIGH EFFICIENCY PARTICULATE AIR (HEPA) FILTERS**

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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<sub>50</sub>). 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<sup>5</sup> 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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pathogens, airborne, spores, aerosol, filtration, viral, infectious, influenza

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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<sub>50</sub>). 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<sup>5</sup> viable plaque-forming units

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

31

32

### **Introduction**

33

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

47 countries, including the United States, had extensive bioweapons programs (Gronvall  
48 2005, Frischknecht 2003). Perhaps the most feared was that of the Soviet Union. Human  
49 history is littered with many examples of microbes being deployed as acts of war and  
50 terrorism, the most recent documented example being the attack on the Hart Building in  
51 2001. This single act of bioterrorism clearly demonstrated the potential threat that  
52 biological agents pose as a weapon of terror.

53

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

65

66 HEPA filters are commonly used in individual and collective protection applications and  
67 are very efficient at removing particulate matter from the air. They are rated to be 99.97%  
68 efficient at collecting the nominal most-penetrating particle (0.3  $\mu\text{m}$ ) (Lee 1980).

69 Although this collection efficiency is impressive, it is not absolute; depending on

70 conditions, 0.03% of matter at the most penetrating size does penetrate the HEPA filter.  
71 For most applications the HEPA is adequate, but tolerance for viral penetration is very  
72 low, and thus only a few penetrating virions may be enough to cause disease. For viruses  
73 to be efficient at penetrating HEPA filters they must remain as submicron particles. Most  
74 agree that viruses will not occur as singlets when dispersed in an aerosol; rather, they will  
75 agglomerate or attach to inert materials that will increase the particle sizes (Stetzenbach  
76 1992). It is important to note, however, that many of the threat agent viruses (*e.g.*, SARS,  
77 EEV) can be significantly agglomerated and still fall into the most-penetrating range.  
78 Most of the research on bioaerosols has focused on naturally occurring biological  
79 aerosols. The research has demonstrated that a majority of particles in biological aerosols  
80 are greater than 1 $\mu$ m in size (Stetzenbach 1992), and thus would not be a threat to  
81 penetrate HEPA filters. It should be noted that the technology used in these studies is not  
82 able to effectively measure bioparticles smaller than 500 nm. Therefore, the abundance of  
83 particles that would be most efficient at penetrating HEPA filters was not properly  
84 quantified. Studies of naturally occurring particulate aerosols (non-biological)  
85 demonstrate that nanometer-size particles are actually abundant (Biswas and Wu 2005).  
86  
87 Weaponized viruses are clearly different from naturally occurring biological aerosols and  
88 the particle size for viral weapons is not clearly defined. From a weapons standpoint, it  
89 would be advantageous to create smaller particles, because they would remain  
90 aerosolized longer. But in addition to creating small particles one must preserve the  
91 viability of the viruses. The methods used to produce and protect viruses from  
92 environmental stress may dictate creating larger particles. It is unclear if weaponized

93 viruses have been created that are submicron in size. This uncertainty has fueled  
94 speculation that viruses may indeed be a threat to penetrate HEPA filters.  
95

96 The study of viral penetration of HEPA filters dates back to the development of HEPA  
97 filters by the Department of Energy (DOE) in the 1950s (Mack, 1957). Since that time  
98 more than 20 published studies have used a variety of experimental techniques to  
99 quantify viable penetration of HEPA filters. A comprehensive review of these studies  
100 edited by Wander is due to be published in 2010. Six studies (Decker 1963, Harstad  
101 1967, 1969, Roelants 1968, Thorne 1960, and Washam 1966) were published in the  
102 1960s; all were chamber tests aimed at determining the viable filtration efficiency of the  
103 media and/or devices. The most elegant of these studies were carried out by Harstad, who  
104 observed that the principal route of penetration is filter defects (pinhole leaks, media  
105 breaks due to pleating, etc.) and not through the medium itself. The next 30 years  
106 produced only eight research articles, six chamber tests (Bolton 1976, Dryden 1980, Eng  
107 1996, Leenders 1984, Rapp 1992, and Vandenbroucke–Grauls 1995), and two studies that  
108 used an animal model (Burmester 1972, Hopkins 1971) to assay the protection provided  
109 by HEPA filters. The turn of the 21<sup>st</sup> century saw a renaissance of interest in research on  
110 viral penetration of HEPA media—a total of seven articles were published in seven years.  
111 Research on active processes for air purification (reactive/antimicrobial media, heat,  
112 energetic light, etc.) that kill microbes rather than just capture them was the main driver  
113 for these studies (Heimbuch 2004, Lee 2008, Ratnesar 2008, and RTI 2006). Dee *et al*  
114 (2005, 2006<sub>a</sub>, 2006<sub>b</sub>) also performed three studies using a swine model to determine the  
115 effectiveness of HEPA filters



116

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

132

133

### **Materials and Methods**

134

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

139 37°C/220 rpm. Lysozyme (Sigma, L6876) was added to a final concentration of 50  
140 µg/mL and the flask was incubated for 30 minutes at 37°C. Chloroform (0.4%) and  
141 EDTA (.02 M) were then added and the culture was incubated for an additional 30  
142 minutes at 37°C. Cell debris was removed by centrifugation at 10,000 X g, then the  
143 supernatant was filtered through a 0.2-µm filter and stored at 4°C. A single-layer plaque  
144 assay was performed according to standard procedures (EPA) to determine the MS2 titer,  
145 which typically is ~10<sup>11</sup> plaque-forming units (PFU)/mL. For aerosol studies, the MS2  
146 coli phage was diluted in either sterile distilled water or 0.5% tryptone to a concentration  
147 of ~10<sup>8</sup> PFU/mL.

148

149 **Aerosol Methods:** The BioAerosol Test System (BATS, Figure 2) is a port-accessible  
150 aerosolization chamber communicating with a temperature/humidity-controlled mixing  
151 plenum and thence to a sampling plenum supplying a homogeneous aerosol to six  
152 sampling ports. Three six-jet Collison nebulizers (BGI Inc, Waltham, Mass.) deliver  
153 droplets at the source that are ~2 µm mass median diameter into the mixing plenum to  
154 create the bioaerosols. Air is drawn into a central vacuum line along a path from the  
155 sampling plenum through lines of PVC tubing (Excelon® RNT, US Plastics, Lima,  
156 Ohio). Each path runs through a test article and thence through one AGI-30 all-glass  
157 impinger (Chemglass, Vineland, N.J.) filled with 20 mL of 1X phosphate buffer  
158 saline/0.001% antifoam A (Sigma, A6457). The volume of air passing in each path is  
159 controlled by a rotameter (Blue–White 400, Huntington Beach, California, or PMR1-  
160 101346, Cole–Parmer, Vernon Hills, Illinois). At the end of the sampling path, the air  
161 exhausts through a conventional HEPA filter and the vacuum pump that drives the air

162 movement. Each sampling port is able to accommodate test articles as large as 6 inches  
163 (15 cm) in diameter.

164

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

177

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

185

186 
$$\text{Total PFU} = \text{counted PFU} \times \text{dilution}^{-1} \times \text{impinger volume}$$

187

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

197

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

202

203 
$$\text{Face velocity (cm/sec)} = \text{flow rate (cm}^3\text{/sec)} \div \text{surface area (cm}^2\text{)}$$

204

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

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

211

## 212 Results

213

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

226

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

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

244

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

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

258

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

266

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

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

281

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



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

301

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

315

316

### Discussion

317

318 Data presented in this report conclusively demonstrate that viable viruses can penetrate  
319 HEPA filters. This should not be surprising given the fact that HEPA filters are rated to  
320 be only 99.97% efficient at collecting 0.3- $\mu\text{m}$  particles. Hence, given a sufficient  
321 challenge, penetration is a mathematical expectation. The penetration is small relative to

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

337

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

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

352

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

367 accounted for  $\sim 1/2$  the total time tested. This indicates that an average male can inhale  
368 particles at velocities greater than the rated velocities for HEPA filters.

369

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

389

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

407

408

### **Summary**

409

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

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

434

435

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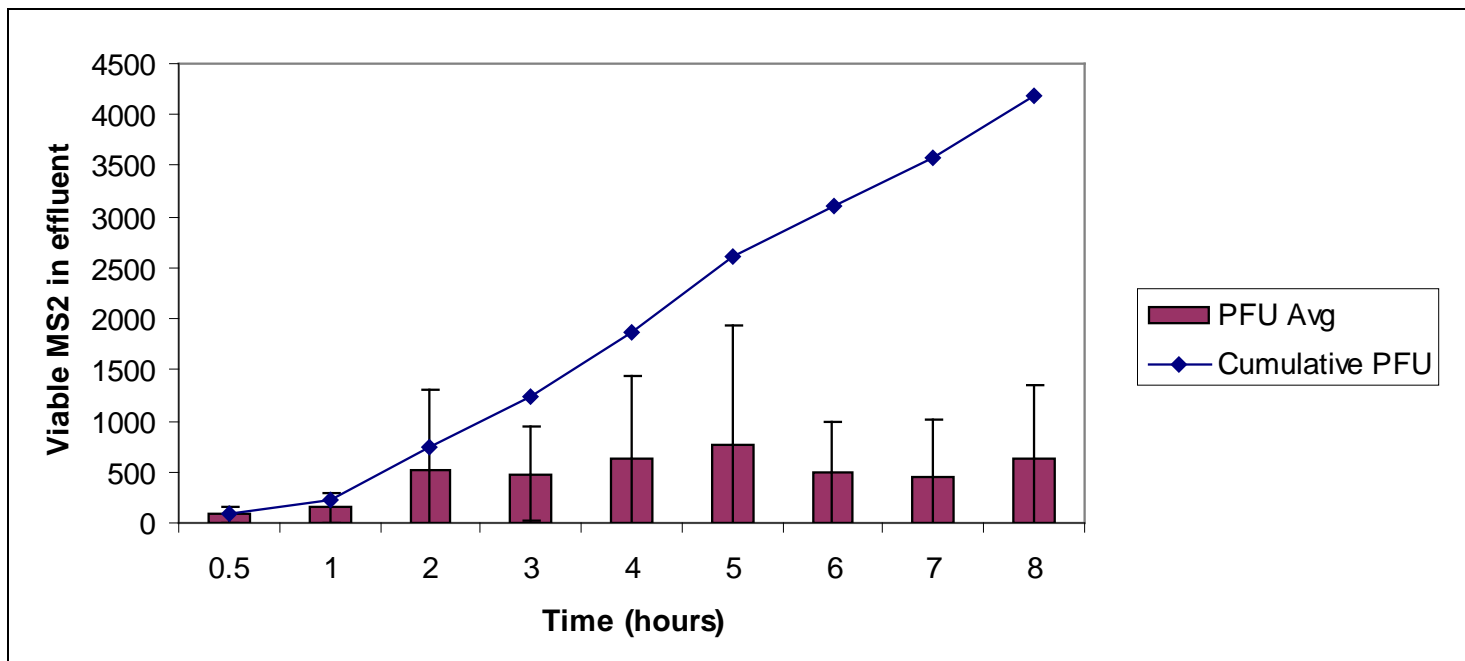


Figure 1: MS2 challenge ( $10^3$  -  $10^7$  PFU/L of air at 85 LPM) of CHAC (n= 21) in BATS

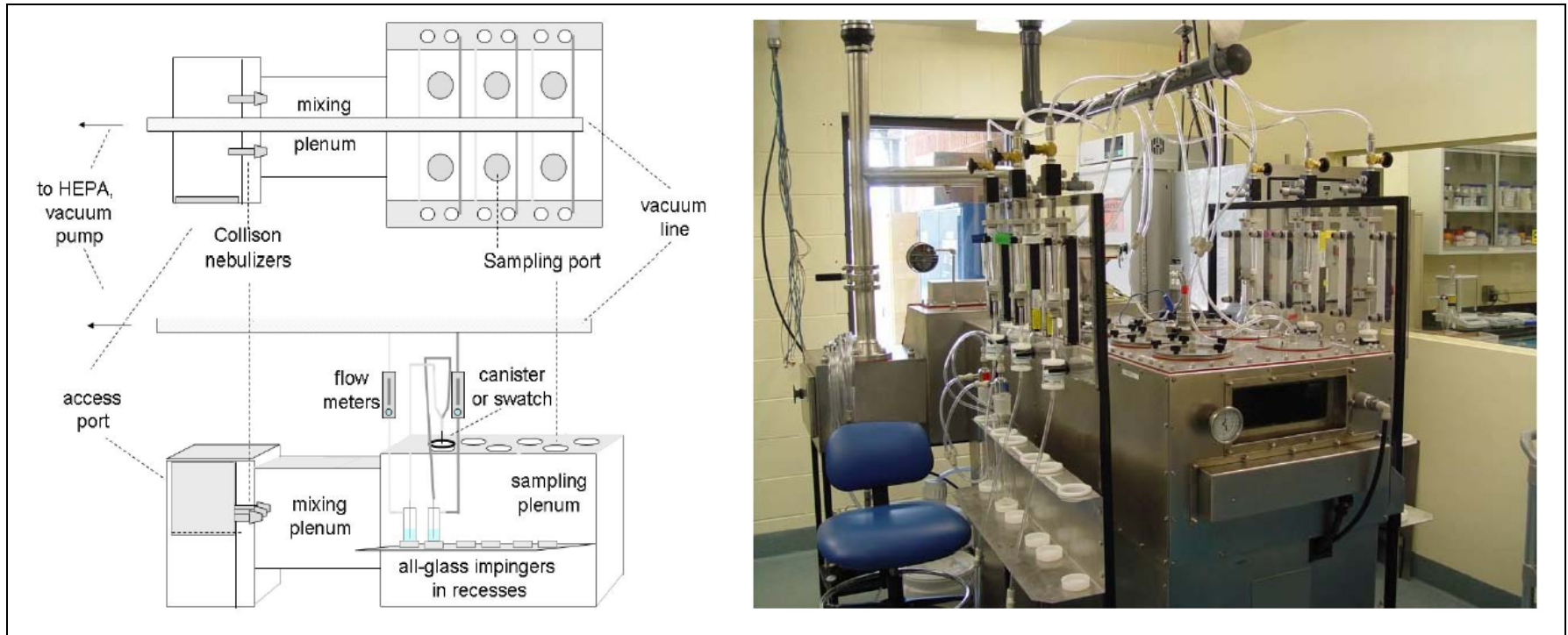


Figure 2: The BioAerosol Test System (BATS) is a Port-Accessible Aerosolization Chamber That is Capable of Safely Generating and Containing BSL-2 Biological Aerosols.

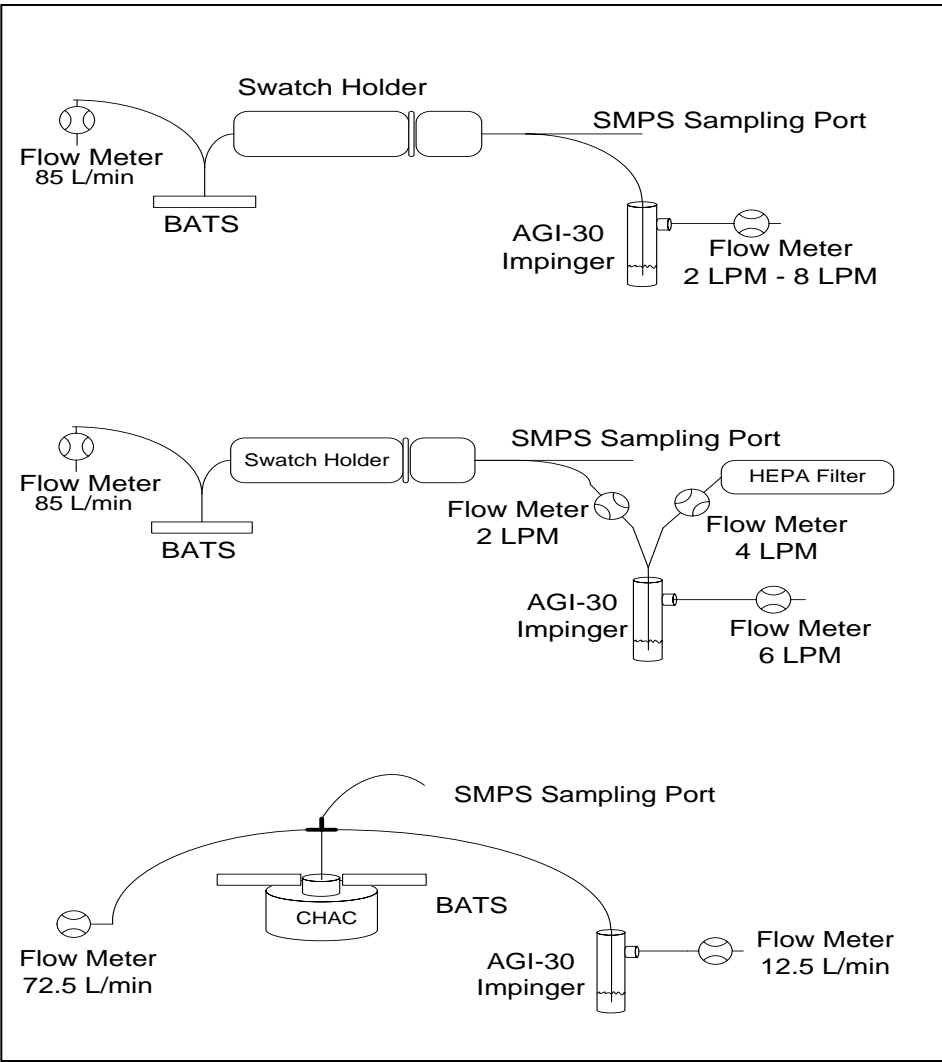


Figure 3: Three Test Configurations for Challenging Flat-sheet HEPA Material and CHACs with MS2 Coli Phage: The overall design allows for airflow downstream of the test article both to be analyzed by the SMPS and to be Collected in an all-glass impinger, allowing for assessment of viable penetration. 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.

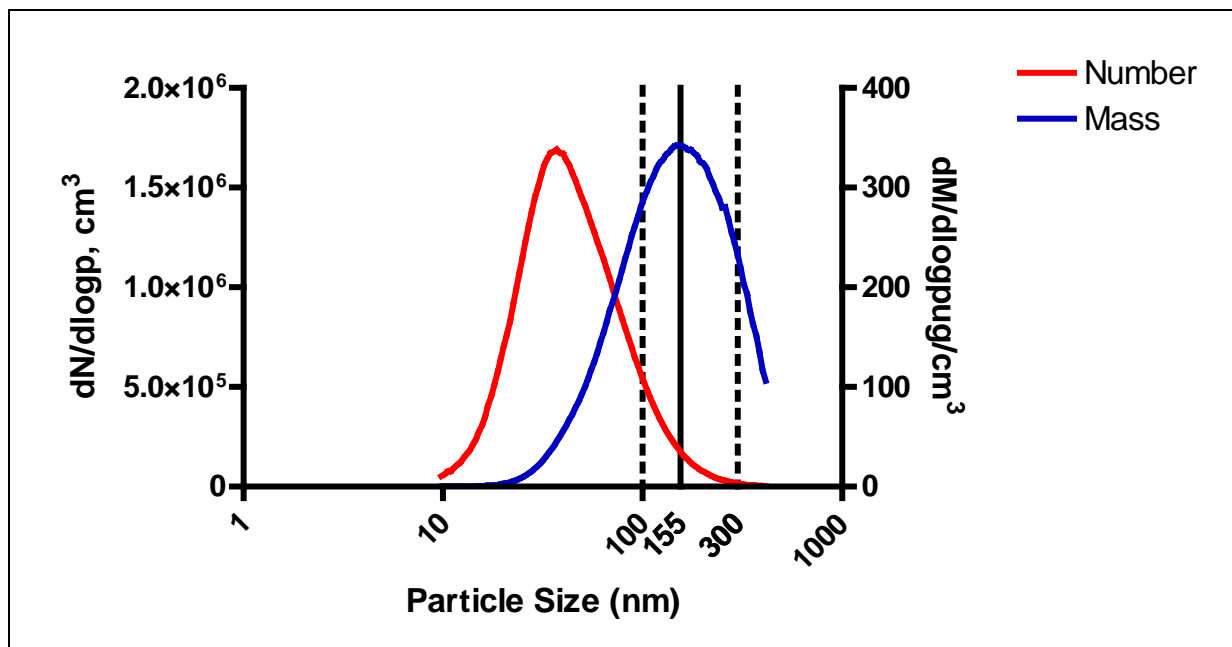


Figure 4: SMPS Analysis of MS2 Aerosolized in Water Using the BATS



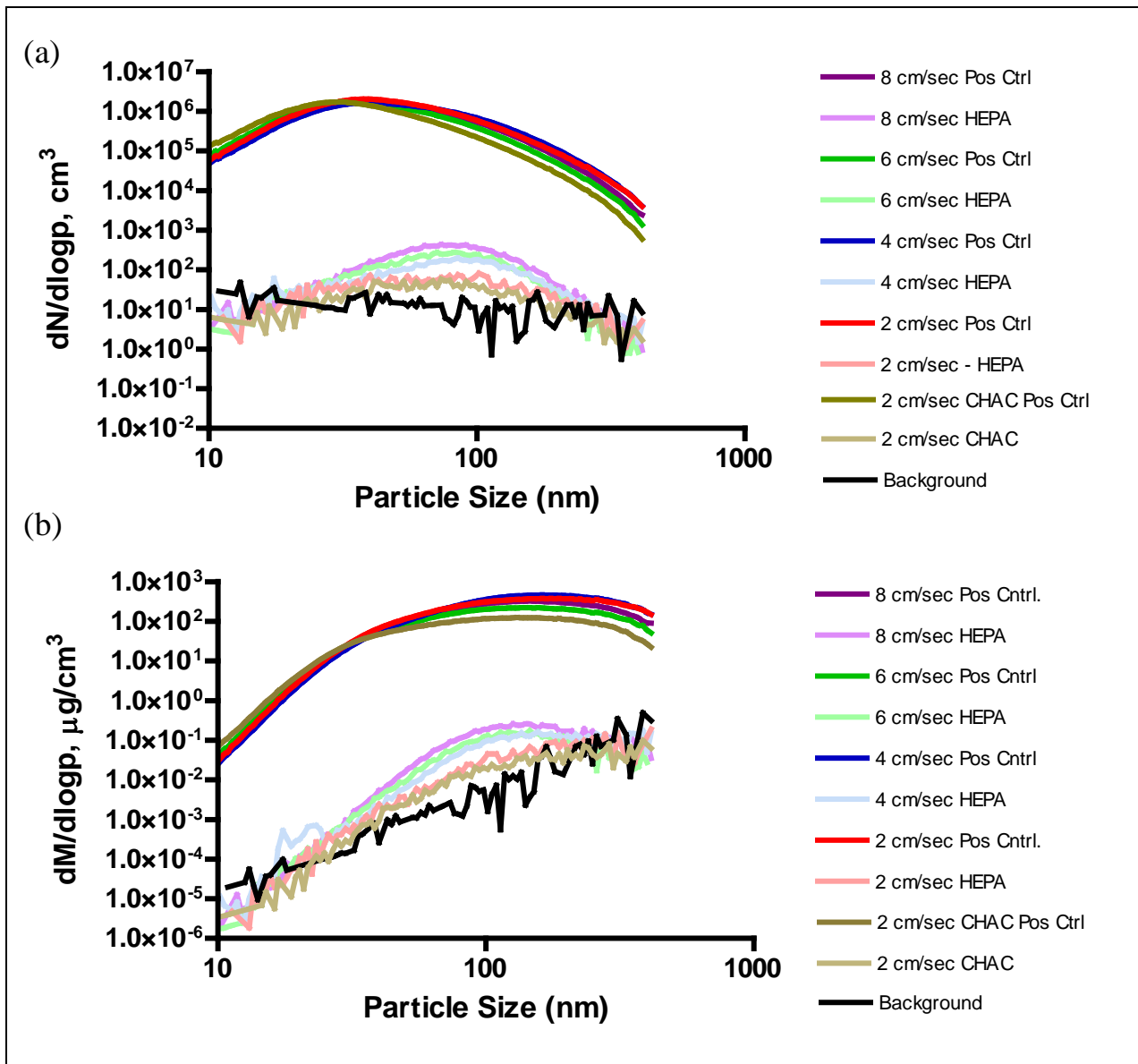


Figure 5: SMPS Analysis of MS2 Coli Phage Challenge of Flat-Sheet HEPA and CHAC [(a) Number, (b) Mass]

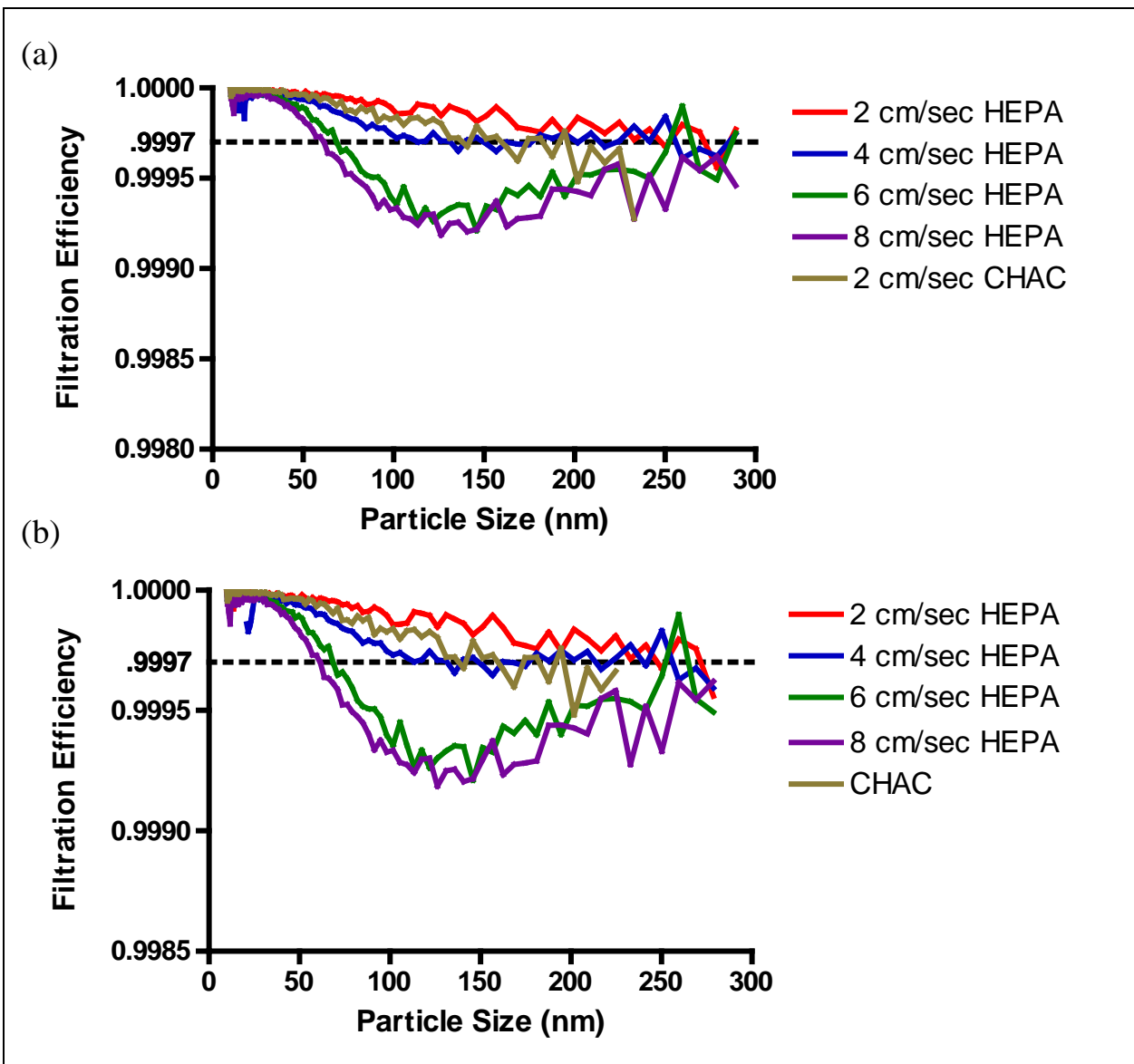


Figure 6: Filtration Efficiency of Flat-Sheet HEPA Challenged with MS2 Coli Phage [(a) Number , (b) Mass]

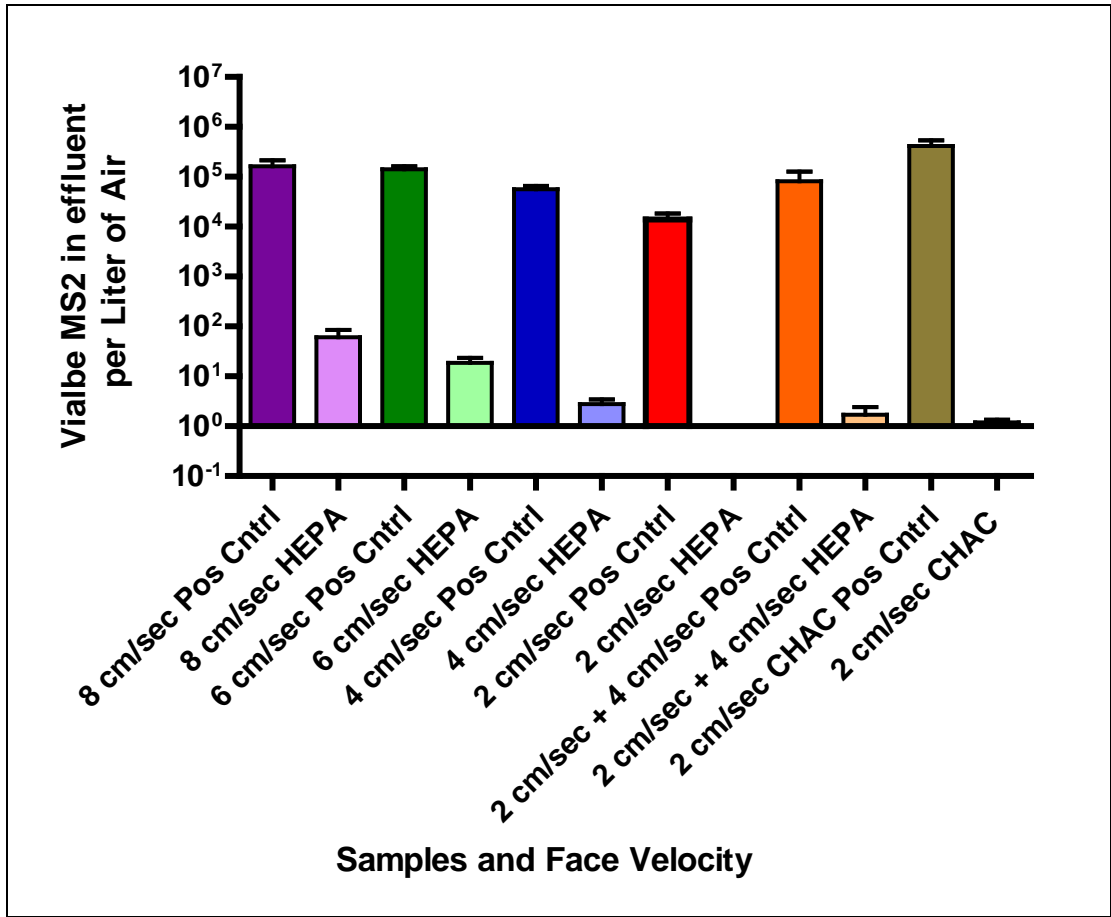


Figure 7: MS2 Challenge of Flat Sheet HEPA and CHAC—Viable Enumeration

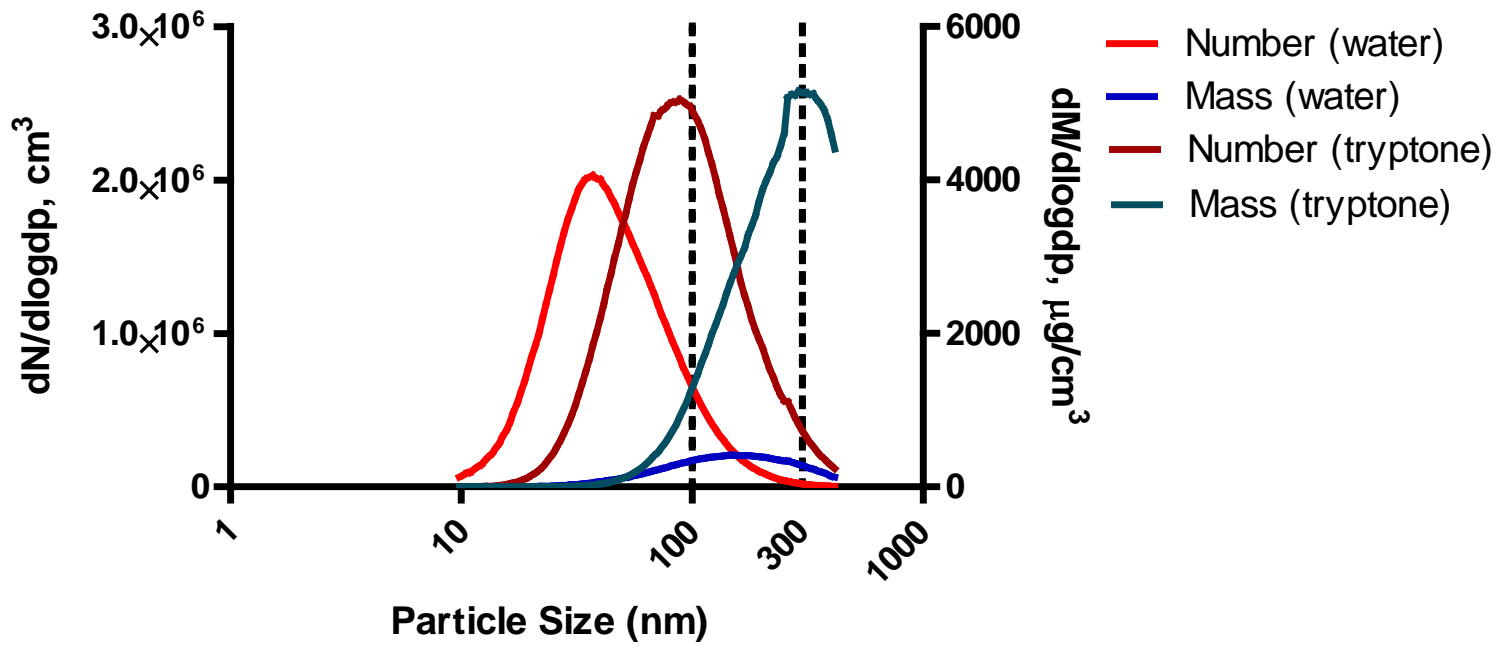


Figure 8: SMPS Analysis: Filtration Efficiency of Flat-Sheet HEPA Challenged with MS2 Aerosolized in 0.5% Tryptone and Water

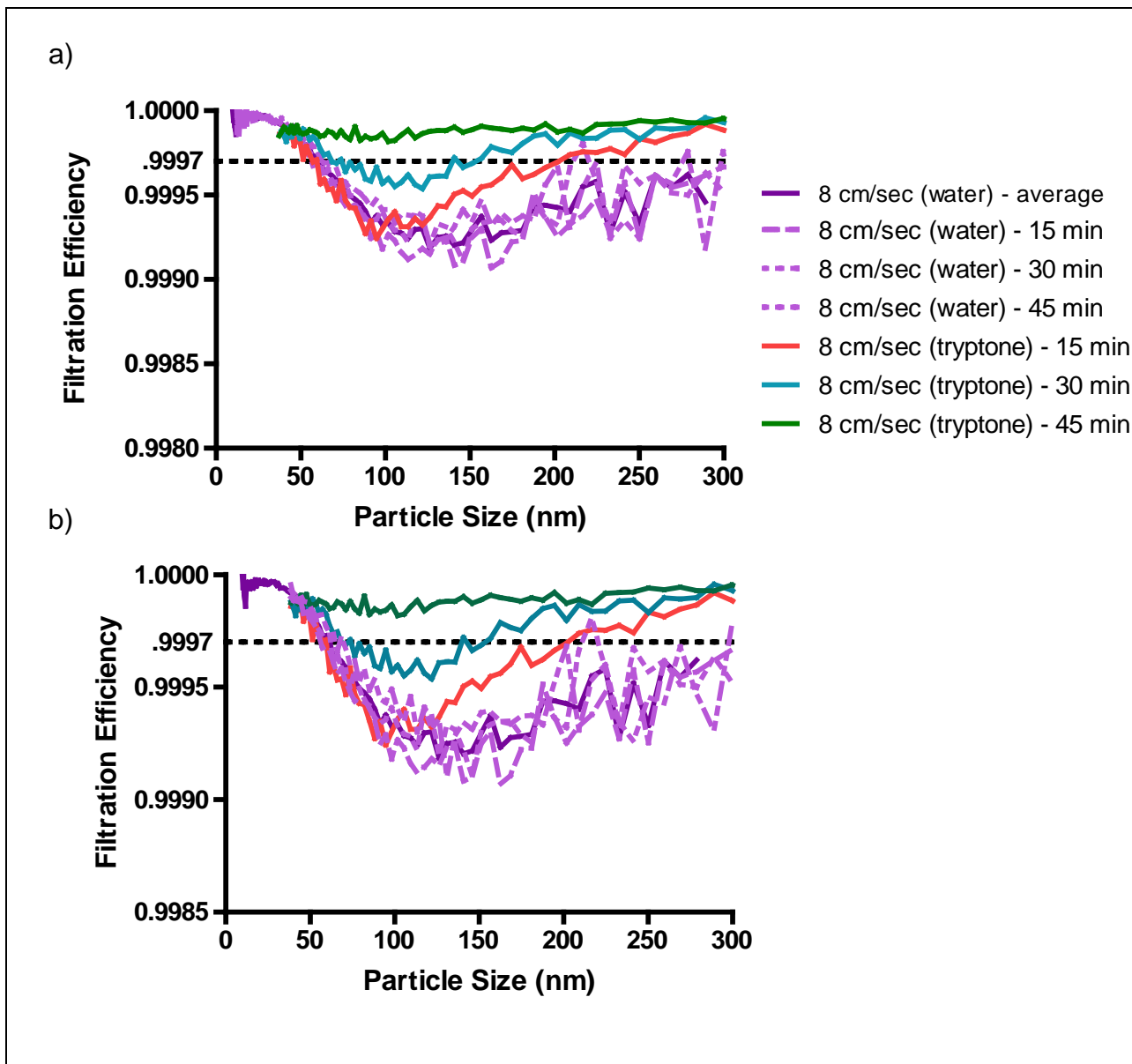


Figure 9: SMPS Analysis of Flat-Sheet HEPA Challenged with MS2 Aerosolized in 0.5% Tryptone and Water

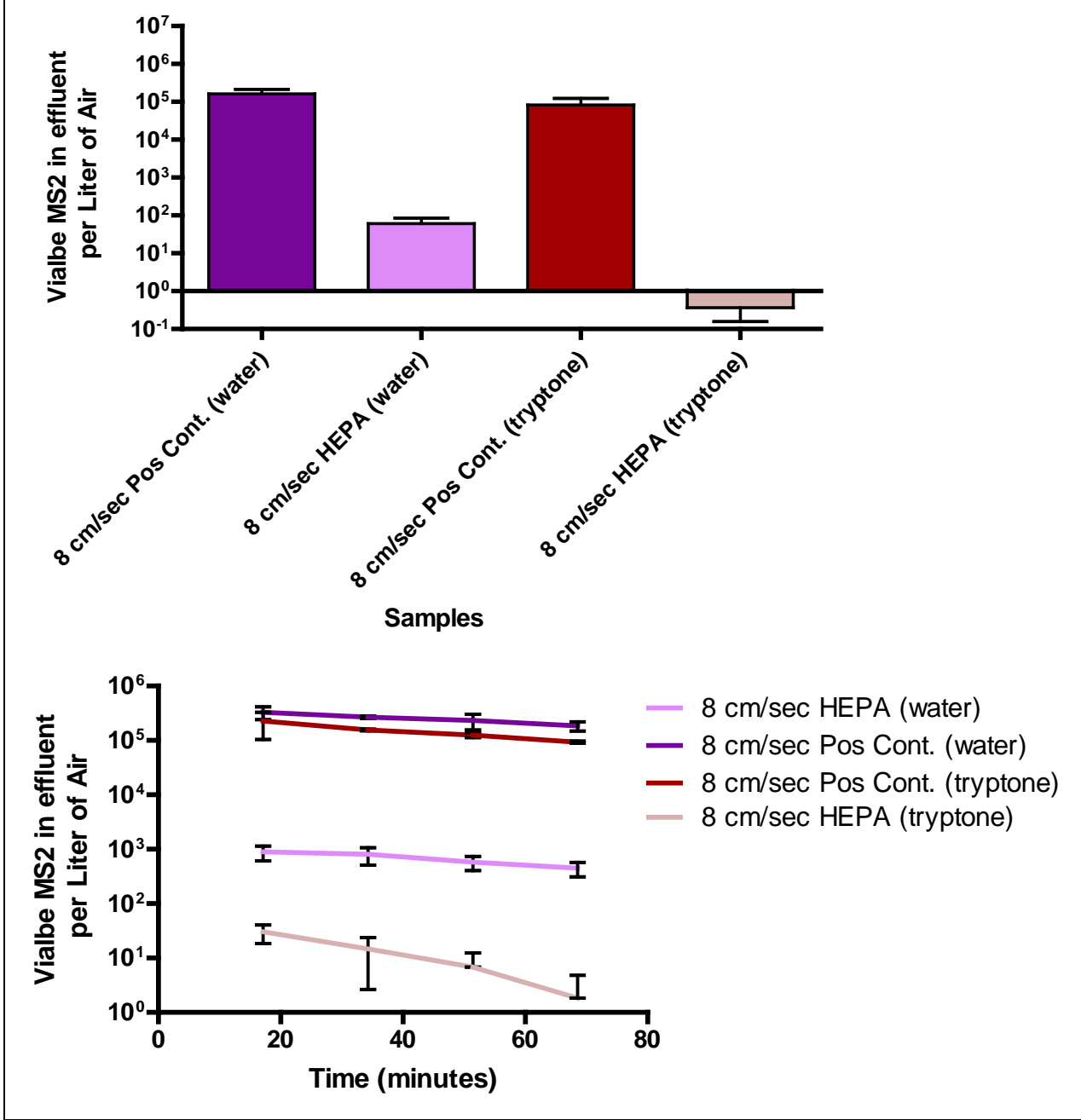


Figure 10: Viable Enumeration of Flat-Sheet HEPA Challenged with MS2 Aerosolized in 0.5% Tryptone and Water

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

Particle Size Diameter	Number Distribution		Mass Distribution*	
	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)