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Evaluation of personal inhalable aerosol samplers with different filters for use during anthrax responses

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Abstract

Risk of inhalation exposure to viable *Bacillus anthracis* (*B. anthracis*) spores has primarily been assessed using short-term, stationary sampling methods which may not accurately characterize the concentration of inhalable-sized spores reaching a person's breathing zone. While a variety of aerosol sampling methods have been utilized during previous anthrax responses, no consensus has yet been established for personal air sampling. The goal of this study was to determine the best sampler-filter combination(s) for the collection and extraction of *B. anthracis* spores. The study was designed to (1) evaluate the performance of four filter types (one mixed cellulose ester, MCE (pore size = 3 μm), two polytetrafluoroethylene, PTFE (1 and 3 μm), and one polycarbonate, PC (3 μm)); and (2) evaluate the best performing filters in two commercially available inhalable aerosol samplers (IOM and Button). *Bacillus thuringiensis kurstaki* [Bt(k)], a simulant for *B. anthracis*, served as the aerosol challenge. The filters were assessed based on criteria such as ability to maintain low pressure drop over an extended sampling period, filter integrity under various environmental conditions, spore collection and extraction efficiencies, ease of loading and unloading the filters into the samplers, cost, and availability. Three of the four tested collection filters—except MCE—were found suitable for efficient collection and recovery of Bt(k) spores sampled from dry and humid as well as dusty and clean air environments for up to 8 hr. The PC (3 μm) filter was identified as the best performing filter in this study. The PTFE (3 μm) demonstrated a comparable performance, but it is more expensive. Slightly higher concentrations were measured with the IOM inhalable sampler which is the preferred sampler's performance criterion when detecting a highly pathogenic agent with no established "safe" inhalation exposure level. Additional studies are needed to address the effects of environmental conditions and spore

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concentration. The data obtained in this investigation are crucial for future efforts on the development and optimization of a method for assessing inhalation exposure to *B. anthracis*.

Keywords

Bacillus anthracis; filter collection; inhalable sampling; viable spore detection

Introduction

The assessment of inhalation exposure to viable *Bacillus anthracis* (*B. anthracis*) aerosolized spores is critical in understanding pathogenicity in the respiratory tract. This issue was highlighted during the 2001 anthrax attacks where *B. anthracis* spores were distributed through the U.S. mail and during naturally occurring events (e.g., when contaminated animal hides were used in drum making and playing). While a variety of aerosol sampling techniques have been evaluated and deployed by different agencies for collecting viable, airborne *B. anthracis* spores during responses and as part of laboratory studies,[1–8] no consensus sampling and analytical method has yet been established for detection of viable spores using personal air sampling devices. When investigators use multiple sampling devices and laboratories utilize multiple analytical techniques, data comparison across contaminated sites becomes difficult if not impossible. Additionally, not all air sampling methods accurately assess risk of inhalation exposure to spores. For example, viability is critical in determining risk of infection and efficacy of decontamination efforts and is therefore preferred for public health evaluations over the detection of DNA via PCR. The air sampling process involves collection of spores through a sampler inlet, transport to a medium such as a filter, or impinger fluid and collection by the medium in a manner that maintains viability over the course of the sampling period. The air sample processing method involves recovery of spores from the collection medium and the determination of the spore concentration. Rigorous performance evaluation is needed to characterize the collection and extraction efficiencies, limits of detection and quantification, and performance variability before a particular collection device and processing procedure can be selected for a multi-laboratory validation study. Significant progress has been made over the past decade to address concerns over the lack of validated environmental sampling methods for *B. anthracis* spores.[9] There are presently two validated laboratory processing procedures available to the Laboratory Response Network (LRN) members for non-porous, surface sampling devices, as well as one standardized laboratory processing method for a porous surface sampling device. While all three are applicable for the detection of viable *B. anthracis* spores, they assess surface contamination and cannot accurately determine what may be inhaled during aerosolization related to specific activities and procedures. Models that attempt linking surface sample results to inhalation exposures have numerous uncertainties.[10,11]

To assess risk of inhalation exposure to pathogenic bioaerosol particles and subsequent infection related to specific aerosol-generating activities, investigators need to know if the particles are viable, if they are in the inhalable size range, and if they reach the personal breathing zone (PBZ). It is important to note that particle size is the major determinant of

where the inhaled particles deposit. The intact spores of *B. anthracis* and their agglomerates can deposit in the upper and the lower respiratory tract potentially causing human infections of different disease profiles.[12] To assess the relevant exposure and dose, inhalable personal aerosol samplers (capable of a particle size selective sampling from the PBZ) have been widely used in occupational settings.[13,14]

Considering the high cost and safety requirements for testing with *B. anthracis* spores, several non-pathogenic *Bacillus* species have been used in the past as surrogates, including endospores of *B. atrophaeus* a.k.a. *B. subtilis* var. *niger*, a.k.a. *B. globigii* (Bg).[5,6,15–17] Alternatively, spores of *Bacillus thuringiensis kurstaki*, Bt(k), have been increasingly utilized as a simulant of *B. anthracis*.[18,19] The endospores of Bt(k) are oval in shape with a length of 1.61±0.18 μm and a width of 0.80±0.07 μm; the hydrated diameter of Bt(k) spores range from 0.59–0.96 μm.[20] These characteristics are similar to a pathogenic *B. anthracis* Ames spore, which is 1.52±0.19 μm long, 0.81±0.06 μm wide, and has a hydrated diameter of 0.40–1.00 μm. At the same time, the dimensions of a Bt(k) spore, as well as a *B. anthracis* spore measurably exceed those of *B. atrophaeus*.[20] For these reasons, Bt(k) was chosen for this study.

Among several commercially available personal inhalable aerosol samplers, the following two are most commonly used in occupational settings: the Institute of Occupational Medicine (IOM) Sampler and the Button Sampler (SKC Inc., Eighty Four, PA). In both devices, aerosol particles are collected on 25-mm filters. Among various commercially available collection filters, the best candidates for measuring airborne spores were selected using criteria such as the ability to maintain low pressure drop over an extended sampling period and sustain the filter integrity under a variety of environmental conditions, high physical collection efficiency for spores, high efficiency of spore extraction procedures, and the ability to easily load and unload the filter in the samplers. Other factors that may become important during large-scale responses include cost and availability. Based on these criteria and data from previous studies,[2–7,21] the following four filters were selected for further evaluation: MCE (pore size = 3 μm), two PTFE (pore sizes = 1 and 3 μm), and PC (pore size = 3 μm) (all from Millipore Corporation, Billerica, MA). The performance of these filters has been characterized with *B. subtilis*;[5,6] however, no similar testing was conducted with Bt(k) spores.[19]

It is acknowledged that the variety of available aerosol samplers (personal and stationary) includes less expensive and, perhaps, easy-to-use models such as a disposable 37-mm cassette that is still widely deployed in the field. However, the latter (similar to several others) has not been specifically designed as an inhalable sampler, which raises a question about utilizing its data for assessing inhalation exposures and developing evidence-based guidance during responses.

Thus, the objective of this study was to evaluate the performance of two inhalable aerosol sampling devices, the IOM and the Button samplers, with four types of 25-mm collection filters for collecting viable Bt(k) spores. The ultimate goal was to identify the best-performing sampler-filter combination(s) for further evaluation. The collected data will ultimately lead to the development of an accepted field collection method coupled with a

validated laboratory processing method for assessing inhalation exposure to viable *B. anthracis* spores.

Materials and methods

The four commercially available 25-mm aerosol collection filters labeled as MCE (3 μm), PTFE (1 μm), PTFE (3 μm), and PC (3 μm), were tested in an approximately 90-L chamber with transparent Plexiglas walls. The chamber was housed in a Biosafety Level II cabinet (Sterilchem-GARD, Baker Co., Sanford, ME).

The first phase of the study was devoted to the evaluation of filters. Each filter (one at a time) was taken from a sterile box and loaded intoa plastic, open-faced filter cassette equipped with a rubber O-ring (Delrin Filter Holder, SKC Inc.). The cassette was placed inside the chamber on a special holder. Each filter was connected to an air pump (Air Diagnostics and Engineering, Inc., Harrison, ME) and tested at two sampling flow rates, 2 and 4 L/min (these are the operational rates for the IOM and the Button samplers, respectively). The tested sampling time was 1, 2, 4, 6, and 8 hr. The volumetric flow rate for each pump, with the sampler and membrane in place, was calibrated before each run using a mini-Buck calibrator (A.P. Buck, Inc., Orlando, FL). The pressure drop through the filter, the filter collection efficiency, the efficiency of spore extraction from the filter, and other performance characteristics were determined for each set of conditions from at least three replicate tests. Based on these data, the filters were ranked aiming at selecting the top performers to be used in the second phase of the study, which examined the performance of the filters loaded in the IOM and Button samplers.

The Bt(k) spores, obtained from the U.S. Department of Defense (product # SA-11 SDTC; technical grade concentrate developed for the U.S. Army and Air Force), were aerosolized from suspension produced by suspending dry Bt(k) spores in sterile deionized water. The spores were washed by centrifugation twice in sterile deionized water at 7,000 rpm to purify the suspension. An initial concentration of ~10^5 to ~10^6 endospores per mL of the solution was established and verified using a hemocytometer (Hausser Scientific, Horsham, PA) before each experiment. If clumping was identified with the hemocytometer, the initial solution was agitated using a 3-min vortexing and re-examined before use. The prepared suspension was transferred to a 6-jet Collison-type air-jet nebulizer (BGI Inc., Waltham, MA) deployed to generate the bioaerosol into the chamber at 6 L min^{-1} after mixing the output airflow with pre-conditioned air of a specific relative humidity (either RH≈20–25% representing dry indoor air conditions or RH≈80–85% representing humid ambient air). The humidity was monitored by a direct-reading thermohygrometer (Fisher Scientific International Inc., Hampton, NH). The nebulizer-generated particles (many of which contained spores from the suspension) were substantially larger than 1 μm; however, shortly after the aerosolization, water evaporated, and the dry spores were transported further toward the filter. The latter was evident from a real-time measured particle size distribution which featured a peak slightly below 1 μm upstream of the filter.

The pressure drop through the tested filter was measured in each experiment using a differential pressure gauge (Model 2080, Dwyer Instruments, Inc., Michigan City, IN) with

an accuracy ±0.5-in water gauge. These measurements were conducted during 8 hr with 1-hr increments at two sampling flow rates (2 and 4 L min^{-1}) and two humidity levels (RH≈20–25% and RH≈80–85%).

To determine the physical collection efficiency of the filters, we measured the upstream (C_{up}) and downstream (C_{down}) concentrations using a real-time aerosol spectrometer (Grimm Technologies, Inc., Douglasville, GA) operating with a new Grimm-proprietary Nanocheck software (version 1.365). For the purpose of this investigation, we recorded the aerosol concentration data for the following six size fractions (indicated by their midpoints): 0.54, 0.615, 0.675, 0.750, 0.900, and 1.15 μm. The collection efficiency was calculated as

$$E_c = \left[1 - \left(C_{down}/C_{up}\right)\right] \times 100\% .$$

For each sampling time interval tested (1, 2, 4, and 8 hr), the collection efficiency was determined as an average of 10 consecutive 1-min measurements.

After collection, the spores were extracted from the filter for the subsequent culture-based analysis. The collection filter was placed in a 50-mL sterile centrifuge tube containing 5 mL of 0.05% Tween-20 (Sigma Aldrich Inc., St. Louis, MO) in water. The tube was vortexed for 2 min using a touch vortex mixer (Fisher Scientific, Inc., Pittsburgh, PA), then placed in an ultrasonic bath (42 KHz Fisher Ultrasonic Cleaner, Fisher Scientific Inc.) for 15 min following a previously established protocol.[5,6] An additional evaluation of shorter intervals was conducted to quantify the effect of ultrasonication time. For this purpose, the entire procedure was implemented with the ultrasonic bath operating at 3 and 7 min instead of 15 min. Each of the three tests was conducted in three replicates, and the resulting culturable counts were compared to determine whether laboratory processing time could be shortened.

Subsequently, the spore suspension was serially diluted in water and 100 μL aliquots of each dilution were spread on tryptic soy agar (TSA) plates in triplicate. The plates were incubated at 37°C for 16–24 hr, the colony forming units (CFUs) counted, and the total CFU per sample calculated. The culturable concentration of the aerosolized spores was calculated by dividing the total number of CFUs per sample by the sampled air volume.

To assess the efficiency of spore extraction from the collection filter after sampling, two consecutive extractions were performed and the data were compared with the single-extraction data. The first extraction resulted in a culturable spore count of CFU_1. The second produced CFU_2. Ideally, at the extraction efficiency (EE) of 100%, CFU_2=0. The EE value was calculated using a previously validated approach[22] as follows:

$$EE = \left\{1 - \left[CFU_2/\left(CFU_1 + CFU_2\right)\right]\right\} \times 100\% .$$

An ultrasonication time of 15 min was used in these tests for consistency.

In the next phase of testing, additional experiments were conducted in the presence of Arizona Road Dust (ISO 12103–1, A1 test dust, Powder Technology, Inc., Burnsville, MN),

which was generated by a specially designed particle disperser at a high mass concentration of ~10 mg m^{-3} simulating extensive dust contamination of the ambient air environment, and ~0.1 mg m^{-3} that represents a typical moderately high indoor pollution.[23–25] This was done to examine worst case scenarios that could increase the pressure drop across the filter due to excessive loading. The dust generator outlet was placed in a close proximity to the Collison nebulizer outlet to enhance the mixing of dust particles with spores in the air. The dust particle size was primarily 1–20 μm.

The filter performance study results were summarized by ranking the filters on a scale of 1–5 with a 5 being the best score for each of the following characteristics:

- pressure drop through the filter at low RH (~20–25%);
- pressure drop through the filter at high RH (~80–85%);
- physical integrity of the filter;
- filter collection efficiency;
- filter extraction efficiency;
- option to reduce the ultrasonication processing time for spore extraction from the filters;
- consistency of performance over the sampling times up to 8 hr;
- data variability;
- ability to manipulate the filter (e.g., easy loading and unloading in the inhalable samplers, absence of major electrostatic issues, filter maintains its integrity);
- consistency of performance in the presence of dust; and
- cost and commercial availability of large quantities on a short notice (may be required during an emergency response).

The best performing filters, based on the results from the ranking, were loaded into the IOM and Button samplers operating at their respective sampling flow rates of 2 and 4 L $min,^{-1}$ and tested in parallel while exposed to bioaerosol concentrations in the range of ~10^5–10^6 CFU m^{-3} (~10^{-1} − 10° CFU cm^{-3}).

Data analysis

SPSS Statistics 17.0 (SPSS Inc, Chicago, IL) was used to analyze the data. The arithmetic mean and the standard deviation of the measured culturable airborne spore concentrations were calculated for each sampling time, collection filter and sampling flow rate. The effect of ultrasonication time was tested by comparing the culturable counts averaged in three replicates using ANOVA. The bivariate correlation analysis was utilized to evaluate the effect of sampling time on the measured culturable spore concentration for both flow rates; the Pearson coefficient was calculated. The univariate analysis of variance was used to examine the effect of collection filter on the concentration of airborne spores recovered. The same analysis was deployed to investigate the effect of the sampling flow rate on the culturable spore concentration for all filters. Unpaired two-tale t-test was utilized to examine

the difference between the two samplers when operating with the best performing filter(s). P-values less than 0.05 were considered to denote significant differences.

Results and discussion

Pressure drop

Measured under typical indoor conditions (RH≈20–25%), the pressure drop through each of the four filters was relatively low at both flow rates: 3.5–6.5 inch w.g. at 2 L min^{-1} and 6.0–14.0 inch w.g. at 4 L min^{-1}. The tests conducted at 1-hr increments showed no pressure drop changes over a sampling time of up to 8 hours (within the measurement accuracy of ± 0.5-in w.g.). As expected, the filter collection at 4 L min^{-1} produced pressure drop approximately twice higher than at 2 L min^{-1}. The PTFE (1 μm) filter featured the highest pressure drop as compared to the other three filters, primarily due to its smaller pore size (1 vs. 3 μm). Collection at higher air humidity (RH≈ 80–85%) produced generally similar results, except for a fragile MCE filter that appeared damaged after sampling in a number of experiments. Even during the shortest sampling time of 1 hr, tears in the filter were observed and some filters were entirely torn apart. The damage of the MCE filter likely created a leak resulting in a lower pressure drop of 3-in w.g. When collecting from a humid air at 4 L min^{-1}, the pressure drop became moderately time-dependent (approximately 20% change was detected over 8 hr).

The pressure drop values observed in this study were substantially below those specified for a long-term continuous operation of commonly used personal sampling pumps such as BGI400 (Mesa Labs, Butler, NJ) (18 inch w.g.) or Leeland Legacy (SKC, Inc.) (20-in w.g.). The measurement results were consistent with the previously reported pressure drop data[6] for the respective filters loaded in the Button Sampler, except for the MCE filter which was not investigated in the cited study.

Physical collection efficiency of filters

Figure 1 displays the results obtained at sampling flow rates of 2 and 4 L/min and RH≈20–25%; the results are shown only for sampling time of 1 hr because no measurable changes in the collection efficiency were observed for 2, 4, 6, and 8 hr. All four filters achieved high collection efficiencies in the spore size range tested. Even the worst case, the PTFE (3 μm) at 4 L/min, the efficiency exceeded 90%. It is acknowledged that the spores may be aerosolized and/or re-aerosolized in clusters (agglomerates) as well as being attached to larger (often non-biological) particles. Therefore, the actual particle size may significantly exceed the single spore diameter. For larger particles, the filter physical collection efficiency increases with an increase in particle size;[26] thus, the single spore-based collection efficiency serves as a conservative estimate.

The physical collection efficiency data were generally consistent with the earlier findings for Bg spores,[5,6] especially for the MCE and PTFE (1 μm) filters. As to the remaining two filters, the first quoted paper reported E_c = 63.6 ± 32.3% for PTFE (3 μm) and E_c = 61.4 ± 24.6% for PC (3 μm), but the second one found E_c to be close to 100% for the same filters. The differences may be attributed to the following: Burton et al. (2005)[5] placed the filters

in the Button Sampler with a metal back-up pad and two plastic O-rings, while the current study used a plastic, open-faced filter cassette equipped with rubber O-rings to prevent leakage and create uniform deposition on the filter face. Also, the version of the Button Sampler cassette used in the quoted study[5] might not have allowed for a proper filter seal, especially with filters as thin as the PC (3 μm) and PTFE (3 μm) filters. This suggests that some leakage may have occurred in those tests which may explain the high variation reported by the authors. Other potential factors contributing to the different collection efficiencies between the studies include a slight variation between filters acquired from different manufacturers and the slight difference in particle sizes of Bg vs. Bt(k).

Collection of aerosol particles by a non-electret filter at higher RH may enhance the filter deposition process and make the filter more efficient.[27] In an extreme case (a wet filter), the efficiency of mechanical filtration increases.[28,29] Thus, we believe that the data obtained at RH≈20–25% represent a conservative case with respect to the physical collection efficiency of the tested aerosol collection filters. An experiment conducted with PTFE (3 μm) and PC filters at RH≈80–85% and 4 L/min confirmed this expectation: 98% and >99% of spores, respectively, were collected by these filters at higher humidity.

Spore extraction from the collection filters after sampling

The bulk of the spores were found to be readily removable from all the four tested collection filters using the conventional extraction procedure described in the Materials and Methods section. The extraction efficiency data are presented in Figure 2. The MCE (3 μm) filter showed the lowest relative spore removal among the four filters (88.3 ± 7.5%); PTFE (1 μm) produced a level of 96.2±2.9%; the two others showed the most efficient extraction (97.9 ± 2.2% and 98.6 ± 1.75%, respectively).

As indicated above, ultrasonication is a part of spore extraction procedure. Previous studies[5,22] used an ultrasonication time of 15 min. However, we found that applying shorter ultrasonication time intervals such as 3 and 7 min did not significantly change the resulting culturable count for three of the four collection filters as compared to 15 min ($p > 0.05$, $n = 3$ each). A longer sonication procedure was found slightly advantageous only for the PTFE (3 μm) filter, but it was not statistically significant. This finding has a practical relevance because it may allow the overall time of analysis to be reduced.

Effect of collection time on spore recovery

The culturable airborne spore concentrations measured using the “standard” protocol, which included a single extraction and a 15-min ultrasonication, are presented in Figure 3 as a function of the collection time for each of the four tested filters at two sampling flow rates. Each data point in this figure represents an arithmetic mean of n measurements. According to the study design, we attempted to collect $n = 12$ replicates for each set of conditions; however, in some cases one or more samples were lost due to low spore viability in the suspension, spore aggregation, improper dilution, contamination, and uncontrolled changes during testing or analysis. In the next step, the role of dilution and the initial spore concentration in the suspension was examined. After demonstrating that neither of the factors had significant impact on the measured concentration of recovered spores, the data

points were combined and averaged, and the corresponding arithmetic mean values and standard deviations of the combined sets were calculated for each sampling time, collection filter, and sampling flow rate. It is concluded that the spore recovery from each of the filters was consistent at sampling times of 1–8 hr. The bivariate correlation analysis conducted for two flow rates failed to reveal any significant effect of the collection time on the measured culturable spores per cm^3 (Pearson coefficient was 0.688). Consequently, all trials conducted with the same filters at a fixed flow rate regardless of the collection time (e.g., 12 + 6 + 11 + 9 = 38 for MCE filter at 2 $Lmin^{-1}$) were treated as replicates and the arithmetic mean of the concentration values measured in all these replicate trials was calculated for each filter and flow rate (these are shown in Figure 3 as horizontal lines). According to Figure 3, the average culturable concentration of the aerosolized spores measured with the four filters fell in a relatively narrow range from 0.84–1.75 CFU cm^{-3} with all but two values being approximately between 1.0 and 1.5 CFU cm^{-3}.

The culturable spore concentration averaged per filter was slightly lower at 4 L min^{-1} than at 2 L min^{-1}; while relatively minor, the difference was statistically significant when analyzed across all tests ($p < 0.05$, univariate analysis of variance). This can, at least partially, be attributed to the observed difference in the physical collection efficiency, especially for PC and PTFE (3 μm) filters (Figure 1).

The effect of collection filter for both sampling flow rates was investigated using the univariate analysis of variance, which failed to identify significant differences between filters with respect to recovery of airborne spores.

Data variability

The variability of the spore concentration data presented in Figure 3 was assessed by the coefficient of variation (CV), which was calculated as a ratio of the standard deviation to the arithmetic mean and expressed in percent (Table 1). The overall variability range was approximately 9–55% with most of CV values falling between 25% and 40%. The main source of the data variability is believed to have been the concentration of spores aerosolized from the suspension, which was directly dependent on the concentration in the suspension (CFU per mL). The latter varied significantly from one experiment to another (at least, by 50% in most cases).

Presence of larger aerosol particles in the sampled air environment

Performance of the collection filters was also studied when, in addition to the airborne spores, the sampled air environment was mixed with dust particles aerosolized using an Arizona Road Dust generator at two concentrations: ~10 and ~0.1 mg m^{-3} (most of experiments were conducted at the latter dust concentration level).

As expected, at ~10 mg m^{-3}, the pressure drop across the filter increased due to loading over time for all the four tested filters (Figure 4). The test was conducted only at 4 L min^{-1} to examine the more conservative case (the pressure drop is lower at 2 L min^{-1}). A moderate increase of the pressure drop was observed for MCE (3 μm), PTFE (3 μm), and PC (3 μm) filters while a much higher pressure drop occurred for the PTFE (1 μm) filter. This is not surprising considering the filter thickness and structure. The observed increased pressure

drop did not significantly affect the filter collection efficiency. This can be partially attributed to the use of a powerful pump, which was able to negotiate the pressure, and, partially, to the advanced filter capacity. Personal air pumps such as BGI400 or Leeland Legacy, which are commonly used for personal air zone sampling, would not be able to operate in a very dusty environment for a prolonged period of time with the PTFE (1 μm) filter (Figure 4 shows the pressure drop exceeding 20 inch w.g. after 2 hr of sampling).

At ~0.1 mg m^{-3}, the pressure drop did not significantly increase, at least over 4 hr (data not shown). The 4-hr period was selected here as a compromise between a short-term and a whole-shift, 8-hr, sampling period.

Figure 5 shows the measured culturable spore concentration obtained with four filters at 4 L min^{-1}, 4 hr, and RH≈20–25% with no airborne dust and with dust at ~0.1 mg m^{-3}. The data suggest that the presence of airborne dust at this level did not significantly affect the filter performance.

Ranking of collection filters

Following the testing results described above we scored and ranked the four filters on a scale of 1–5 with “5” corresponding to the best performing filter and “1” to the worst performing filter. Table 2 presents the scores according to the characteristics listed in the Materials and Methods section as well as the total scores.

Based on the findings with respect to the performance under different conditions, user-friendliness and cost, the MCE (3 μm) and PTFE (1 μm) filters were dropped from further consideration. The PC (3 μm) filter received the highest total score, which is consistent with the earlier reported results.[21] However, it was not singled out as the best because some previous studies suggested that PC filters have a lower spore recovery compared to others such as PTFE.[5,22] Additionally, as availability of products can vary, it is advisable to consider more than one filter type/model. Thus, we selected two filters with the highest total scores, PC (3 μm) and PTFE (3 μm), to be further tested in the IOM and Button samplers.

Performance testing of IOM and Button samplers with PC (3 μm) and PTFE (3 μm) filters

The next phase included the comparison of performance of the IOM and Button inhalable aerosol samplers utilizing the two filters. The samplers were tested in parallel, with the same filter installed, at a sampling time chosen to be 1 hr, which minimized the effect of changes in aerosol concentration over time. Figure 6 presents the concentration measurement results from 10 tests; in this figure, the culturable spore concentration measured with the IOM and the Button samplers were plotted against each other. The tests were performed while spores were generated in the presence of Arizona Road Dust at ~0.1 mg m^{-3} to simulate a field-relevant, conservative case.

It was concluded that the IOM Sampler collected a greater number of spores per the sampled air volume as compared to the Button Sampler. It is also acknowledged that the difference between the results produced by the two samplers is not statistically significant ($p>0.05$, unpaired 2-tales t-test) when used with the PC filter, which moderate the above conclusion

about the choice of one inhalable sampler over the other. At the same time, the effect of a sampler was found to be significant for the PTFE (3 μm) filter.

The same statistical test revealed that for either of the two samplers, the choice of a filter [PC (3 μm) vs. PTFE (3 μm)] does not significantly affect the measurement result.

Limit of detection/quantification

In the context of assessing inhalation exposure of *B. anthracis* spores that may occur in an anthrax response, it is important to be aware of the limit of detection (LOD) and limit of quantification (LOQ) of the measurement method used. For the culture-based bioaerosol analysis, the lowest countable number is 1 CFU per agar plate. In this study, the collection filter was placed in a 5-mL extraction solution and, in case of low spore concentration, a 100 μL aliquot was plated directly (with no serial dilution). Thus, the initial filter sample would need to contain at least 50 culturable spores to generate one colony per plate, assuming 100% extraction efficiency. It is customary to adopt the practical quantification limit (PQL) = 5 × LOD as the lowest level that can be reliably achieved within specific precision and accuracy limitations provided by routine laboratory operating conditions.[30–32] Consequently, the PQL in this study was 250 culturable spores per filter. Assuming an 8-hr sampling, this is achievable if the ambient concentration of culturable spores is at least 130×10^{-6} CFU cm^{-3} (130 CFU m^{-3}) at a sampling flow rate 4 L min^{-1} (Button Sampler) or at least 260×10^{-6} CFU cm^{-3} (260 CFU m^{-3}) at 2 L min^{-1} (IOM Sampler). If one assumed an extraction efficiency of 90%, the actual detection thresholds (representing the PQL) would increase to 143 and 286 CFU m^{-3}, respectively. If the sampling time is shorter or the sampling flow rate is lower, the threshold becomes higher. The protocol used in this study can be modified to develop the capability for assessing air environments containing extremely low concentration of culturable spores. Instead of a 100 μL aliquot, the entire 5-mL volume of the extraction solution can be filtered through a sterile filter, then placed face up on the agar plate and incubated.[17] This 50-fold increase of the volume available for analysis results in a PQL 250/50 = 5 CFU/filter. If sampling for 8 hr at 4 L min^{-1} on a filter allowing 90% extraction, a quantification threshold as low as ≈ 3 CFU m^{-3} can be achieved. Additional studies are needed to develop adequate spore delivery techniques to consistently generate low levels of spores which would allow determining the LOD more accurately.

Study limitations

An accurate quantification of spores captured by a filter is often challenging due to the uncertainty associated with the filter extraction efficiency. The method used in this study to determine the extraction efficiency of the filters was adopted explicitly as a way to compare the relative extraction efficiency of the four filter materials. We recognize that two extractions may not remove 100% of the spores; however, it was found[22] with epifluorescent microscopy that only 2–4% of microorganisms remained on a polycarbonate filter after a single extraction. Therefore, we believe that the fraction remaining after two extractions should not significantly impact the outcome, at least at the tested spore concentration levels. Additionally, this study was carried out in a controlled laboratory environment in absence of other microorganisms, pollen, fibers, etc. The presence of those in the field could complicate the target spore detection. Studies should be conducted to test

the protocol with other microorganisms and particles present at low (near LOD) levels. Also, an investigation of spores remaining on the filter should be included.

Conclusions

Both the IOM and Button filter-based samplers demonstrated an adequate performance for assessing the personal inhalation exposure to aerosolized Bt(k) spores simulating *B. anthracis*. Three out of four collection filters tested in this study (excluding MCE) were found suitable for efficient collection and recovery of Bt(k) spores sampled from dry and humid as well as dusty and clean air environments for up to 8 hr. The relative extraction efficiency of spores from these three filters was at or above 95% regardless of the ultrasonication time period tested (3, 7, and 15 min). Based on multiple criteria, it was concluded that the PC (3 μm) filter was the best performing filter in this study. The PTFE (3 μm) demonstrated a comparable performance, but it is more expensive. The IOM Sampler appeared to be the best option since higher measured concentrations are the preferred criterion of a sampler's performance when assessing risk of inhalation for a highly pathogenic agent such as *B. anthracis* spores given there is no established "safe" inhalation exposure level for viable *B. anthracis* spores. Additional studies are needed to more rigorously assess the performance of the two inhalable samplers under various environmental conditions and a wide range of airborne spore concentrations while the samples are analyzed by multiple laboratories representing variability in processing and culturing the samples. Furthermore, the protocols developed and used in this effort may be utilized to include other aerosol sampling devices, particularly inexpensive disposable samplers that can possibly offer additional practically feasible assessment options, depending on the scale and needs of the response. The data obtained in this investigation are crucial for developing a consensus field collection method and associated validated processing method for assessing inhalation exposure to aerosolized *B. anthracis* spores.

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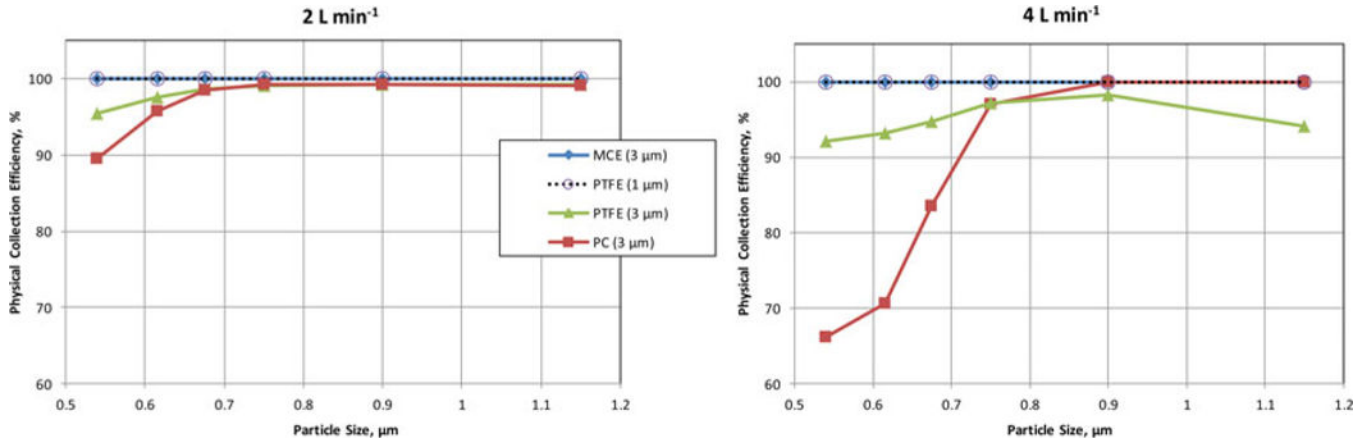

Figure 1.

The physical collection efficiency of four filters at sampling flow rates of 2 and 4 L min^{-1} determined at RH≈20–25%. Each data point represents an arithmetic mean of ten consecutive 1-min measurements. The standard deviations are too small to be seen in the graphs. MCE = Mixed Cellulose Easter, PTFE = Polytetrafluoroethylene, PC = Polycarbonate.

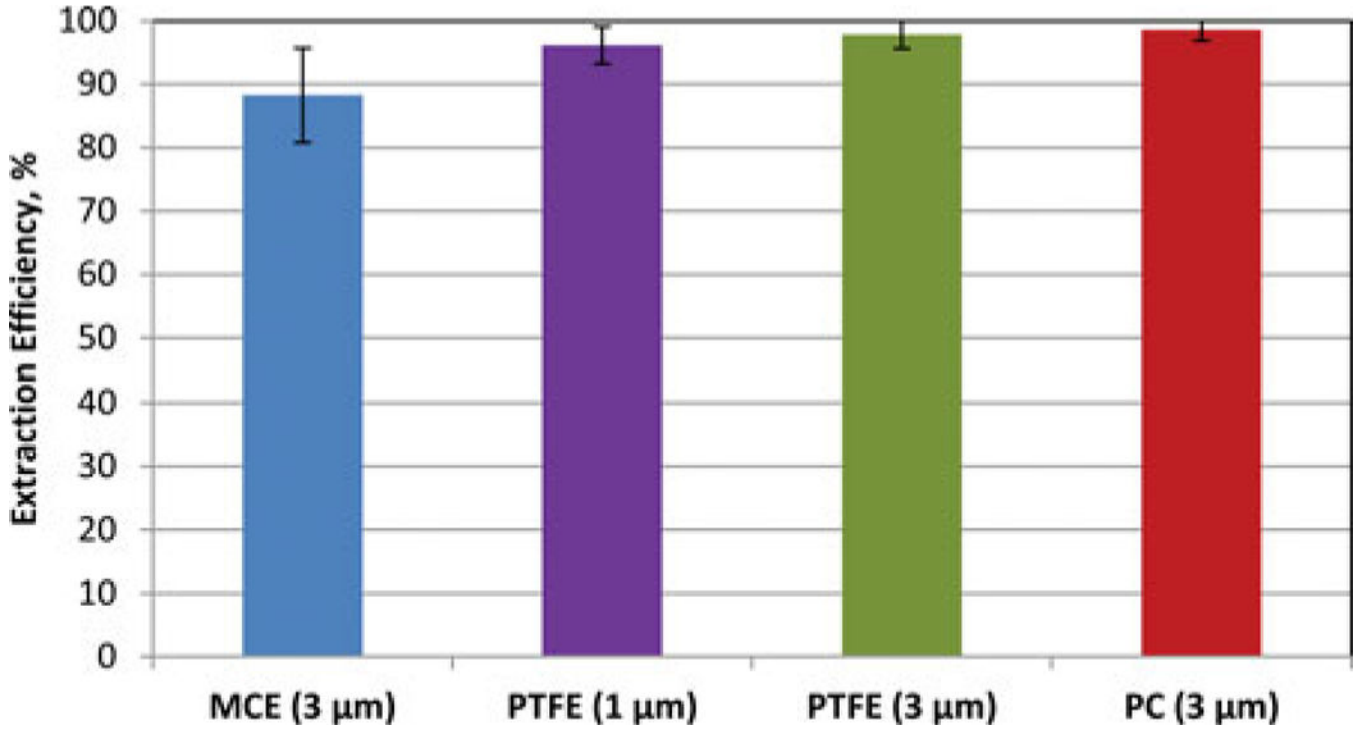

Figure 2.

Relative extraction efficiency (EE) for Bt(k) spores collected on different filters (based on the difference between one- and two-extraction procedures). Time of ultrasonication = 15 min (standard). Each bar represents an arithmetic mean value with error bars representing a standard deviation of at least three replicate measurements.

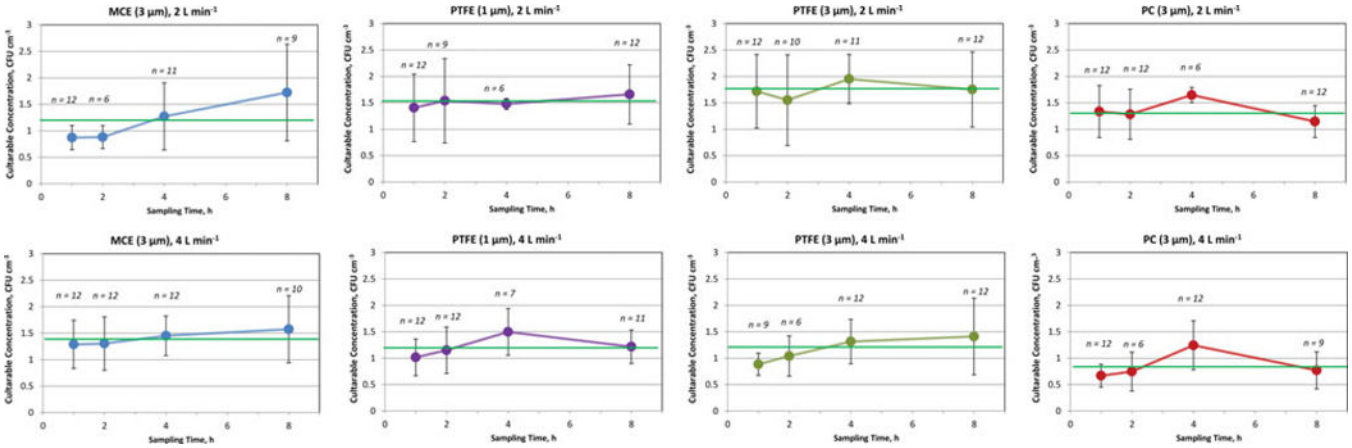

Figure 3.

The measured concentration of culturable airborne Bt(k) spores plotted against the sampling time for four filters collecting at 2 and 4 L min^{-1}. *n* = number of replicate CFU counts. Time of ultrasonication = 15 min (standard). Each horizontal line represents an arithmetic mean of all data points in the graph, including those obtained during 1-, 2-, 4-, and 8-hr collection ($n_{total} = n_1 + n_2 + n_4 + n_8$). The error bars represent the corresponding standard deviation.

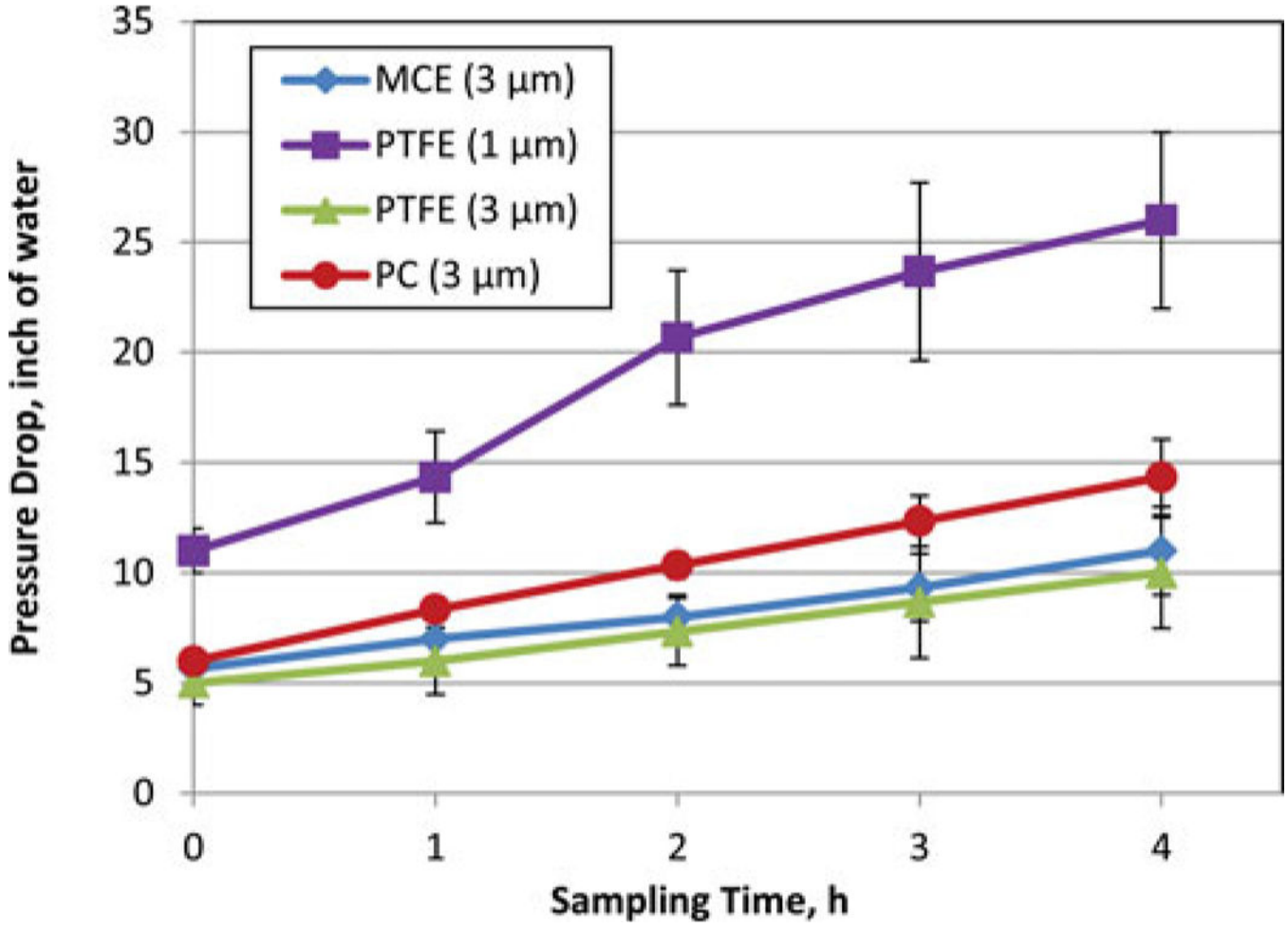

Figure 4.

Pressure drop through the collection filters as a function of sampling time in presence of high level of Arizona Road Dust (~10 mg m^{-3}). Each data point represents an arithmetic mean value with error bars representing a standard deviation of at least three replicate measurements.

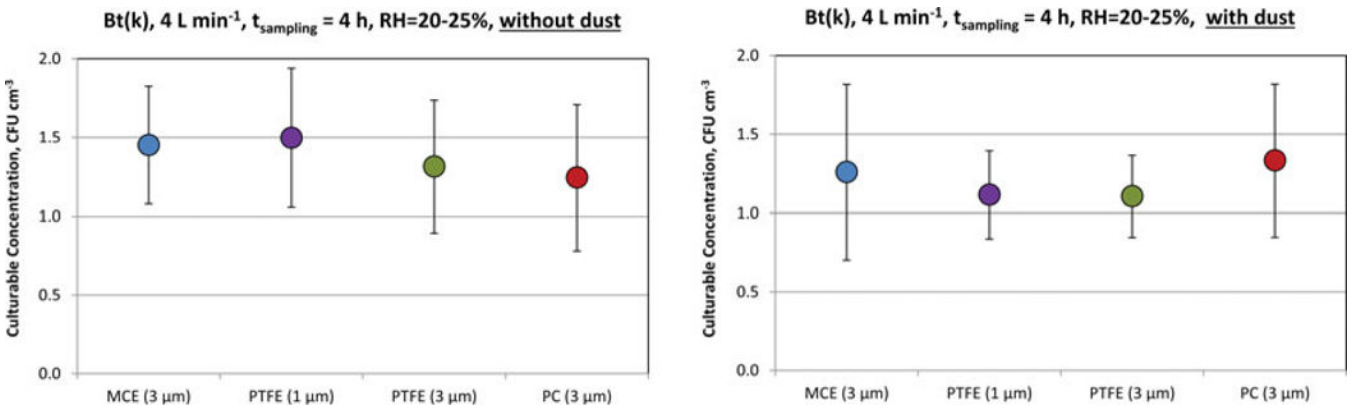

Figure 5.

Culturable concentration of airborne Bt(k) spores collected from different filters without dust in the air and in a moderate presence of Arizona Road Dust (~0.1 mg m^{-3}). Each data point represents an arithmetic mean value with error bars representing a standard deviation of at least three replicate measurements.

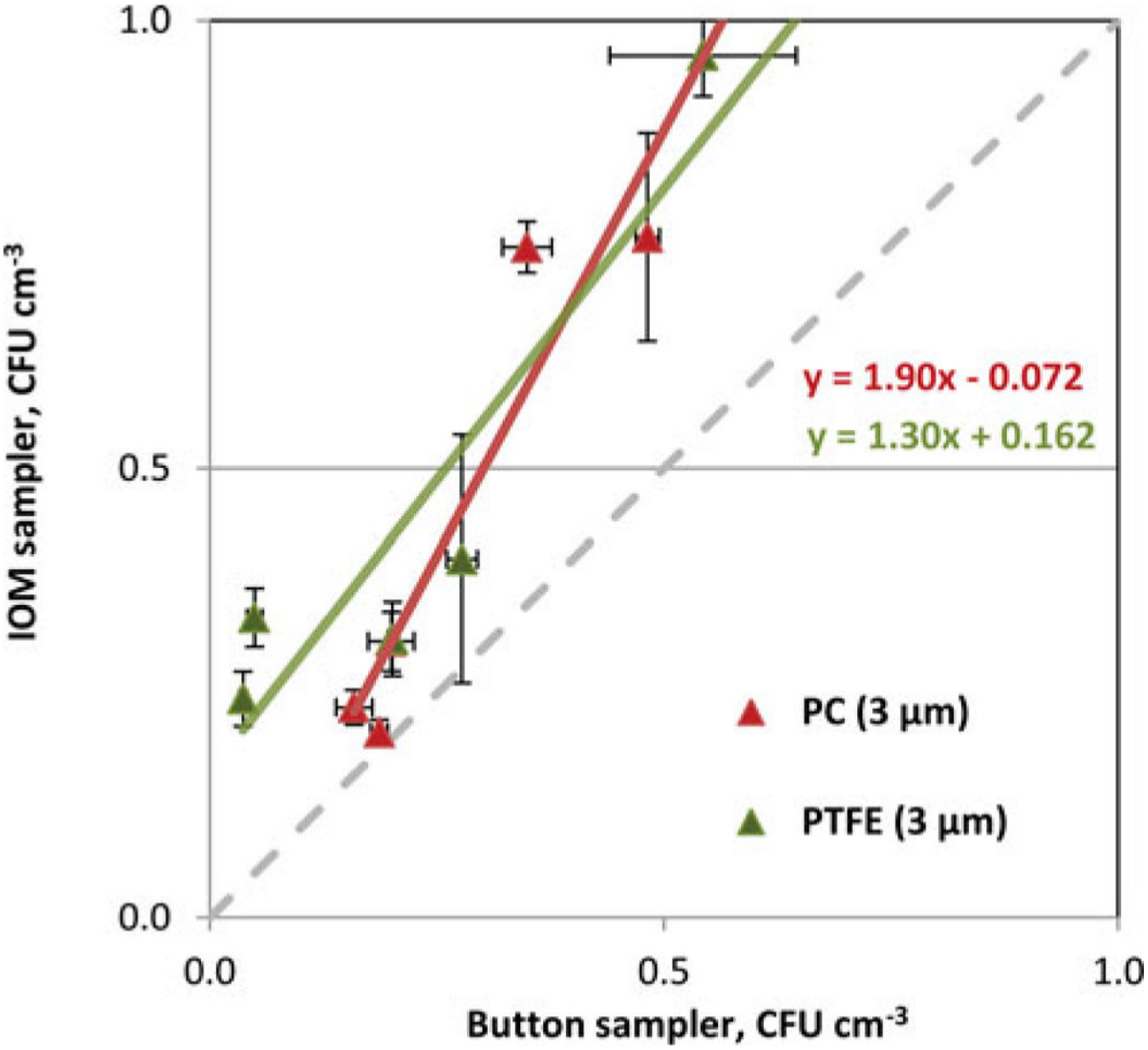

Figure 6.

Culturable spore concentrations measured with the two samplers loaded with two collection filters in a moderate presence of Arizona Road Dust (~0.1 mg m^{-3}). Each data point represents an arithmetic mean value with error bars representing a standard deviation of at least three replicate measurements.

Table 1.

Coefficient of variation = (SD/Arithm.Mean) × 100% for the data presented in Figure 3.

(a) 2 L min^{-1}				
	Coefficient of variation, CV (%)			
Time, hr	**MCE (3 μm)**	**PTFE (1 μm)**	**PTFE (3 μm)**	**PC (3 μm)**
1	26	45	41	37
2	25	52	55	37
4	50	7	24	9
8	53	34	41	26
(b) 4 L min^{-1}				
	Coefficient of variation, CV (%)			
Time, hr	MCE (3 μm)	PTFE (1 μm)	PTFE (3 μm)	PC (3 μm)
1	35	34	24	32
2	39	38	37	49
4	26	30	32	37
8	40	26	52	45

Table 2.

Scores for collection filters.

	Collection filter score from 1 (low) to 5 (high)							
	MCE (3 μm) Flow rate, L min^{-1}		PTFE (1 μm) Flow rate, L min^{-1}		PTFE (3 μm) Flow rate, L min^{-1}		PC (3 μm) Flow rate, L min^{-1}	
Characteristic	**2**	**4**	**2**	**4**	**2**	**4**	**2**	**4**
Pressure drop @ low RH	5.0	5.0	5.0	4.0	5.0	5.0	5.0	5.0
Pressure drop @ high RH	broken		5.0	4.0	5.0	5.0	5.0	5.0
Physical integrity of the filter at high RH	1.0	1.0	5.0	5.0	5.0	5.0	5.0	5.0
Physical collection efficiency in the Bt(k) size range	5.0	5.0	5.0	5.0	4.5	4.0	4.5	4.0
Extraction efficiency	4.0	4.0	4.5	4.5	5.0	5.0	5.0	5.0
Capability of shortening the ultrasonication time	5.0	5.0	5.0	5.0	4.0	4.0	5.0	5.0
Consistency at different sampling times = 0–8 hr	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Total recovery variability (CV)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
User-friendliness of the filter manipulation	4.0	4.0	5.0	5.0	4.0	4.0	3.5	3.5
Consistency of performance in the presence of dust	5.0	5.0	3.0	3.0	5.0	5.0	4.5	4.5
Cost and availability	4.5	4.5	2.5	2.5	3.0	3.0	5.0	5.0
TOTAL per flow rate	**42.5**	**42.5**	**49**	**47**	**49.5**	**49**	**51.5**	**51**
TOTAL average score for two flow rates	42.5		48		49.25		51.25	