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# Comparison of Air Sampling Methods for Aerosolized Spores of *B. anthracis* Sterne

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Bacillus anthracis *Sterne spores were aerosolized within a chamber at concentrations ranging from $1\times10^3$ to $1.7\times10^4$ spores per cubic meter of air (particles (p)/m$^3$) to compare three different sampling methods: Andersen samplers, gelatin filters, and polytetrafluoroethylene (PTFE) membrane filters. Three samples of each type were collected during each of 19 chamber runs. Chamber concentration was determined by an aerodynamic particle sizer (APS) for the size range of 1.114–1.596 μm. Runs were categorized (low, medium, and high) based on tertiles of the APS estimated air concentrations. Measured air concentrations and recovery efficiency [ratio of the measured (colony forming units (CFU)/m$^3$) to the APS estimated (particles/m$^3$) air concentrations] for the sampling methods were compared using mixed-effects regression models. Limits of detection for each method were estimated based on estimated recovery efficiencies. Mean APS estimated air concentrations were 1600 particles/m$^3$, 4100 particles/m$^3$, and 9100 particles/m$^3$ at the low, medium, and high tertiles, respectively; coefficient of variation (CV) ranged from 25 to 40%. Statistically significant differences were not observed among the three sampling methods. At the high and medium tertiles, estimated correlations of measured air concentration (CFU/m$^3$) among samples collected from the same run of the same type were high (0.73 to 0.93). Among samples collected from the same run but of different types, correlations were moderate to high (0.45 to 0.85); however, correlations were somewhat lower at the low tertile (–0.31 to 0.75). Estimated mean recovery efficiencies ranged from 0.22 to 0.25 CFU/particle with total CVs of approximately 84 to 97%. Estimated detection limits ranged from 35 to 39 particles/m$^3$. These results will enable investigators to conduct environmental sampling, quantify contamination levels, and conduct risk assessments of* B. anthracis.

**Keywords** aerosols, bioaerosols, emergency response, recovery efficiency, sampling methods

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

## INTRODUCTION

Air sampling methods for *Bacillus anthracis* were scrutinized following the 2001 incident involving *B. anthracis* letters that were found at various workplaces in the eastern United States. The Centers for Disease Control and Prevention (CDC) published sampling procedures for *B. anthracis* that recommended the use of pumps with filters or impactors.[1] The Government Accountability Office (GAO) subsequently published a report recommending that agencies validate sampling methods for *B. anthracis*.[2]

During the 2001 *B. anthracis* attacks, responders used a variety of air and surface sampling methods. Some air sampling methods used concurrently did not detect *B. anthracis,* in contrast to other air and surface sampling results.[3] McCleery et al.[3] evaluated a postal processing center in Trenton, New Jersey, for *B. anthracis* in 2002 and determined that the Andersen sampler was more sensitive than other sampling methods used in the study [mixed-cellulose ester (MCE), polytetrafluoroethylene (PTFE), gelatin filters, and dry filter units]. Although similar volumes of air were sampled using the different methods, the number of Andersen samples collected was six to eight times higher compared with the other sampling methods.

Sanderson et al.[4] collected air samples using MCE filters at a postal processing center in Washington, D.C., in 2001. Although these samples were negative for *B. anthracis,* surface samples collected at the same time were positive. Weis et al.[5] collected air samples using Andersen and gelatin filter samplers at a U.S. Senate office in 2001. *B. anthracis* was detected with both methods, but concentrations were much higher using the Andersen samplers.

Laboratory studies have used spore simulants to characterize these air sampling methods. Buttner and Stetzenbach[6] conducted a laboratory test using fungal spores of *Penicillium chrysogenum*. They found that Andersen and Burkard samplers were the most accurate compared with surface air system and dispositional samplers and that Andersen samplers had the highest levels of sensitivity and reproducibility. Burton et al.[7] used *B. subtilis* var *niger* to compare filter materials for environmental sampling methods. Physical collection efficiency was found to be 94% or greater for MCE filters, 1-$\mu$m PTFE filters, and gelatin filters, while the 3-$\mu$m PTFE filters showed inconsistent physical efficiency characteristics.

There is no minimum infectious dose or safe aerosol level for *B. anthracis* exposure. Determining infectious dose requires an understanding of the potential for aerosol exposure by humans and the infectivity of inhaled spores. Inhalation infectivity has been researched, but estimates of a lethal dose vary.[8,9] Bartrand et al.[10] conducted a risk analysis on the mortality of guinea pigs and rhesus monkeys exposed to *B. anthracis* spores and found a 50% lethal dose ($LD_{50}$; i.e., the dose at which 50% of subjects die) of about 100,000 spores inhaled for 1-$\mu$m particles. It is difficult to equate inhalation infectivity with a measured aerosol level because of the inability to quantify the following: exposures to the resultant aerosol, uptake by humans, room size and ventilation characteristics, and exposure time. Despite these limitations, it is necessary to evaluate the performance of air sampling methods.

To aid in validating sampling methods for determining airborne concentrations of *B. anthracis* in the event of a biological attack or natural outbreak, research was conducted in an experimental chamber designed to produce targeted concentrations. The goal was to compare selected air sampling methods (Andersen, PTFE, and gelatin, described below) at low airborne concentrations of *B. anthracis* Sterne and estimate recovery efficiencies and limits of detection.

## MATERIALS AND METHODS

### Spore Preparation and Settling Chamber

Spores of *B. anthracis* Sterne, an attenuated strain used in vaccines, were used throughout. Spore preparation has been described in complete detail elsewhere.[11] The settling chamber and aerosol generation system, constructed at Dugway Proving Ground, have been described in complete detail elsewhere.[12] Briefly, the settling chamber was 1.22 m high with a cross-section of 1.22 m by 2.44 m with static-dissipative Plexiglas walls and an extruded aluminum frame.

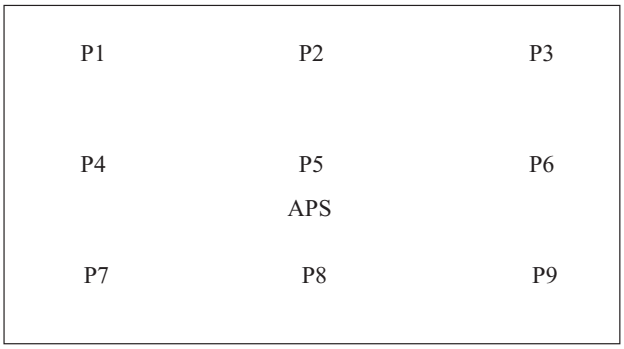


**FIGURE 1**. Diagram of the chamber floor. Sample locations within the chamber were indicated by P1-P9; the APS intake was located at the point labeled "APS." Intake tubes for each sampler were spaced evenly across the chamber at a height of 20 cm. The chamber volume was 3.6 $m^3$ (1.22 m high with a cross-section of 1.22 m by 2.44 m). A 3 $\times$ 3 Latin square design was used to position the three sample types (Andersen, PTFE, and gelatin) in different arrangements for each chamber run.

Dry spore-containing particles were aerosolized into this settling chamber using an aerosol generation system. The aerosol generation system consisted of a Small-Scale Powder Disperser (Model #433; TSI, Inc., Shoreview, Minn.), an impactor (Model #266; Marple Impactor, Sierra Instruments, Carmel Valley, Calif.) to remove particles greater than 5 $\mu$m, and a mixing tunnel with a turbulence induction element propelled by nitrogen dilution gas with an Ion Air Cannon static eliminator (Exair, Inc., Cincinnati, Ohio). The chamber contained nine sampling intake locations evenly spaced within the chamber for the three air sampling methods (described below) and one intake in the center of the chamber for an APS (Model APS 3321, TSI, Inc.) (Figure 1).

### Air Samplers

Three air samples were collected during each chamber run for each of the following air samplers: Andersen N6 single stage impactors (Model 10–709; Thermo-Andersen, Smyrna, Ga.), 1-$\mu$m polytetrafluoroethylene membrane filters (PTFE, #225–1715; SKC, Inc., Eighty Four, Pa.), and gelatin filters (SKC #225–9552). Samplers were assigned to the nine positions using a 3$\times$3 Latin square design (a different design was used for each run of the chamber). Andersen samplers were connected to a critical orifice for a nominal 28.3 liters per minute (L/min) flow rate (in practice, the flow rate averaged 24.8 L/min). Plates of 10 cm diameter trypticase soy agar with 5% sheep blood (TSA II, Becton Dickson Microbiology, Franklin Lakes, N.J.) were used in the Andersen samplers and to process all sampling media (described below). TSA II plates were collected from the samplers using aseptic techniques.

Each N6 sampler was thoroughly sanitized before each use by submerging each inlet port and sieve section in 10% bleach for 10 min and then rinsing with a thiosulfate solution (0.12 M sodium thiosulfate, 19 g/L water). PTFE membrane filters were 37 mm diameter, 1.0 $\mu$m pore size, 60 $\mu$m laminated

support, preloaded in a three-piece clear styrene (traditional style) cassette. Gelatin filters were 25 mm diameter with a 3 $\mu$m pore size and presterilized gelatin (water soluble). Gelatin filters were used with closed-face button samplers (Button Aerosol Sampler; SKC #225–360). PTFE and gelatin filters used low-flow pumps (SKC 224) calibrated (Dry Cal; Bios, Butler, N.J.) at 2.0 L/min before each use. For each chamber run, a control sample for each sampler type was collected by loading the sampler with media and removing it without activating the sampler. A slit sampler was run at 28.3 L/min for 30 min before and after each chamber run to determine the concentration in the room that housed the chamber.

## Chamber Operation

Samplers were loaded with the appropriate sampling media in a biological safety cabinet located in a room separate from the room that housed the chamber. Samplers were placed inside the chamber with caps over the air intakes. Samplers, fans, and chamber walls and surfaces were coated with light oil (WD-40, San Diego, Calif.) to reduce resuspension of particles. The chamber was sealed and purged with HEPA-filtered air for about 30 min (active pumping). Aerosol concentration during chamber operation was monitored in real time (averaging 5 min) with an APS. *B. anthracis* Sterne spores were introduced and aerosolized into the chamber using the generation system described by Baron et al.[12]

Since the initial concentration was much higher than desired, the following process was used to reduce the chamber to the intended concentration: first, the chamber was purged for 20 to 45 min using active pumping; second, the pumps were turned off and the concentration was further reduced by 10 min of stirred settling that entailed running one chamber fan at a time for 1 s/min; finally, the APS-monitored concentration decay was modeled using an exponential decay equation.[12,13] This equation was used to forecast the time at which the chamber would reach the intended air concentration because the number of particles counted by the APS at these very low concentrations was extremely variable and could not be relied on in real time.

Once the forecast time was reached, glove ports were used to open all intake caps and then the sampling pumps were turned on. Gelatin and PTFE low-flow pumps were activated for 60 min. Andersen pumps were activated at times 13, 26, and 46 min for 1.5 min each time to minimize their disturbance of the spore concentration while maintaining a sample volume similar to the other samplers. The Andersen start and stop times were determined using an exponential concentration decay model subject to stirred settling and activation of all nine sampling pumps. By this means, the expected concentration in the chamber during the sampling period was simulated by accounting for the removal of spores arising from the low- and high-flow sampling pumps as well as from particle settling. After the sampling period, pumps were turned off and the samplers were capped using the glove ports. The remaining aerosol in the chamber was removed by purging

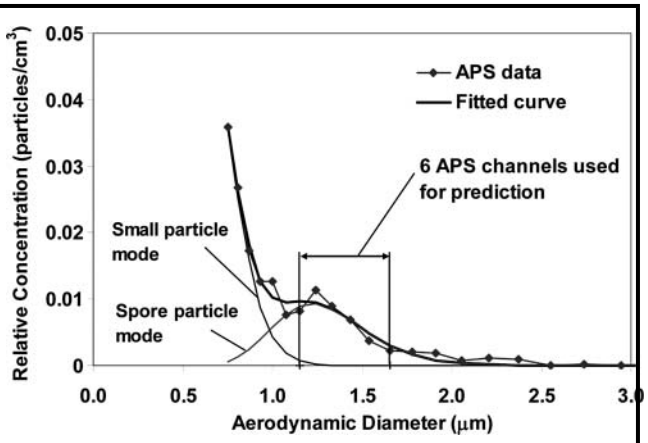


**FIGURE 2**. Example of the curve fitting process described in the text for estimating the total number of single-spore-containing particles from the APS data.

the chamber for 1 hr. Then, all samples were removed and placed in a biological safety cabinet that was located in another room.

The study was designed to include 9, 4, and 6 chamber runs at low (300 particles/m$^3$), medium (1000 particles/m$^3$), and high (3000 particles/m$^3$) air concentrations, respectively. Without exposure limits or other evaluation criteria, there was little on which to base these levels. These levels were selected based on target concentrations used during earlier surface sampling method experiments.[14] More chamber runs were intended to be conducted at the low concentration because of the greater expected variability. In practice, mean APS estimated air concentrations for the 19 chamber runs were higher than intended concentrations because of a calculation error of the exponential decay equation. The calculation error (i.e., the reversal of the dependent and independent variables) resulted in earlier target times and, therefore, higher than expected concentrations. In two runs, the actual concentration also deviated from the intended concentration because of operator error and a power outage.

Consequently, APS values, each taken over 5-min periods, were used to estimate the true air concentration post hoc by fitting two log-normal curves to the APS at diameter, d, (*APS(d)*) for each run (Figure 2). A total concentration of the sum of the log-normal background distribution $B$ and spore distribution $S$ was calculated as a function of diameter $d$:

$$T(d) = C_B \times B(d, \sigma_{gB}, CMD_B) + C_S \times S(d, \sigma_{gS}, CMD_S) \quad \textbf{(1)}$$

where $C_B$ and $C_S$ are the background and spore concentrations to be determined, $\sigma_{gB}$ and $\sigma_{gS}$ were the respective diameter geometric standard deviations and $CMD_B$, and $CMD_S$ were the respective diameter geometric means of each distribution. $S$ and $B$ were of the form

$$F(d, \sigma_g, CMD) = \frac{1}{\sqrt{2\pi}\ln\sigma_g} \exp\left[\frac{-(\ln d - \ln CMD)^2}{2(\ln\sigma_g)^2}\right] \quad \textbf{(2)}$$

such that

$$\int_0^\infty F(d, \sigma_g, CMD) d \ln d = 1 \quad \textbf{(3)}$$

The values $C_B$, $C_S$, $\sigma_{gB}$, and $CMD_B$ were varied until the residuals of $APS(d)$–$T(d)$ were minimized over nine or more diameter values. To improve the convergence and maintain realistic values, the values of $\sigma_{gS}$ and $CMD_S$ were predetermined by measuring their values at the time of dispersion when the spore mode was prominent, and calculating these values at the time of exposure through simulated exponential stirred settling based on the chamber's characteristics. In this way, the spore mode distribution was constrained to be in the expected range. This produced the best estimate of the spore concentration and was named the "APS estimated air concentration" for each run. Resultant concentrations ranged from 1000 to 17,000 particles/m$^3$.

Using the APS estimated concentrations, three tertiles of six or seven runs each were created to recategorize the runs into low, medium, and high tertiles. Nine of the 19 chambers runs moved to different categories (high, medium, or low) from the original target.

## Sample Analysis

Agar plates removed from the Andersen samplers were incubated at 35 to 37°C for 16 to 18 hr, and the resulting *B. anthracis* colonies were enumerated. Two solutions were used for suspending spores collected on the filters: phosphate-buffered saline (PBS, #P3813–10PK; Sigma Aldrich, St. Louis, Mo.) and Butterfield Buffer with Tween 80 (BBT, 0.01%, pH 7.2). PTFE and gelatin filters were placed into centrifuge tubes containing 25 mL PBS with 0.02% Tween 80. Tubes were initially vortexed for 2 min in 10-s bursts, then PTFE filters were removed and discarded. Tubes were vortexed for an additional 30 s and sonicated (40 KHz) for 30 s. The vortexing and sonicating steps were repeated two additional times. Three 8-mL aliquots of suspension with 20-mL BBT were filtered onto 0.45 $\mu$m mixed cellulose ester (MCE, Cat #4800; Pall Corp., East Hills, N.Y.) filters. Each MCE filter was placed onto a TSA II plate. All plates were incubated at 35 to 37°C for 16 to 18 hr, and the resulting *B. anthracis* colonies were enumerated. Many of the gelatin samples, especially from chamber runs at the low target concentration, were not filtered because the solution appeared cloudy and may not have filtered completely. For these samples, the number of colony forming units (CFU) was estimated by plating all sample that could be recovered onto 18 to 26 TSA II plates at 0.6 to 0.8 mL/plate.

## Statistical Methods

All statistical analyses were performed using SAS 9 Software (version 9.2, SAS Institute Inc., Cary, N.C.). Results with p-values less than 0.05 were considered to be statistically significant. Andersen results (CFU/sample), corrected using the positive hole correction factor,[15] were converted to an air concentration (CFU/m$^3$) by dividing the corrected CFU count by the sample volume. The mean pump flow rate for the Andersen samplers was 24.8 L/min. Gelatin and PTFE results were converted to air concentrations by dividing the estimated number of CFUs (based on the mean of three replicate filters, adjusted for the sampling fraction) by the estimated sample volume. The mean pump flow rate for the gelatin and PTFE samplers was 2.00 L/min. The mean of the pre- and post-calibration flow rates was used to convert individual samples; however, the overall mean flow rate was used to convert samples from the first four runs because the calibration data were not available.

Correlations between the APS estimated air concentration (particles/m$^3$, 1 per run) and the measured air concentrations (CFU/m$^3$, 3 repeats per run) were estimated using mixed-effects regression models based on methods described by Hamlett et al.[16] A separate model was fit for each air sampler type (Andersen, gelatin, and PTFE) using the MIXED procedure in SAS. Each model included a random effect for source (APS, measurement) with an unstructured covariance matrix. Concentrations from different runs were treated as independent.

Means of the measured air concentrations (CFU/m$^3$) for the air samplers were compared using mixed-effects regression models. Runs were categorized into tertiles (low, medium, and high) based on the APS estimated concentrations, and a separate model was fit to data from each tertile using the MIXED procedure in SAS. In these models, air sampler type was treated as a fixed effect, and since the nine air samplers were arranged in a 3×3 Latin square design, row and column were included as additional fixed effects. The model specified air sampler type as a random effect (with an unstructured covariance matrix) and a repeated effect (with separate error variances for each sampler type) that resulted in estimates of between-run and within-run variances for each sampler type and estimates of covariance for each pair of sampler types. Measurements from different chamber runs were treated as independent. The model was given by

$$\begin{aligned} y_{ij} = {} & \beta_0 + \beta_1(\text{type}_{ij} = \text{Andersen}) + \beta_2(\text{type}_{ij} = \text{gelatin}) \\ & + \beta_3(\text{type}_{ij} = \text{PTFE}) + \beta_4(\text{row}_{ij} = 1) + \beta_5(\text{row}_{ij} = 2) \\ & + \beta_6(\text{row}_{ij} = 3) + \beta_7(\text{col}_{ij} = 1) + \beta_8(\text{col}_{ij} = 2) \\ & + \beta_9(\text{col}_{ij} = 3) + \alpha_i(\text{type}_{ij} = \text{Andersen}) + \gamma_i(\text{type}_{ij} \\ & = \text{gel}) + \pi_i(\text{type}_{ij} = \text{PTFE}) + \varepsilon_{ij} \end{aligned} \quad \textbf{(4)}$$

where $y_{ij}$ was the measured air concentration (CFU/m$^3$) for run $i$ ($i = 1, 2 \ldots n$) and sample $j$ ($j = 1, 2 \ldots 9$); $\beta_1 = 0$, $\beta_4 = 0$, and $\beta_7 = 0$; random effects $\alpha_i$, $\gamma_i$, and $\pi_i$ were normally distributed with mean zero and an unstructured covariance matrix; and random errors $\varepsilon_{ij}$ were normally distributed with mean zero and a diagonal covariance matrix. Recovery efficiency of the samplers, defined as the ratio of the measured air concentration (CFU/m$^3$) to the APS estimated air concentration (particles/m$^3$), was compared using a similar mixed-effects regression model.

For all mixed-effect regression models, covariance parameters were estimated using restricted maximum likelihood (REML), and degrees of freedom were estimated using the Kenward-Rogers method. Model residuals were approximately normally distributed after three samples identified as outliers were removed from consideration; consequently, a transformation of the dependent variable was not required.

A minimum of one CFU was required to detect *B. anthracis* for each sample; therefore, the minimum concentration of particles in the air that would result in one or more CFU/sample was estimated and called a "limit of detection." That is, the limit of detection was defined as the particle concentration (particles/$m^3$) required to recover at least one CFU based on the estimated recovery efficiencies. This limit of detection was calculated rather than measured directly. Furthermore, this definition assumes that the recovery efficiency at the limit of detection was the same as the recovery efficiency at the concentrations evaluated.

## RESULTS

All laboratory control filters and plates were negative for CFUs. Slit samplers that measured the concentration within the room that housed the chamber averaged 5.0 spores/$m^3$ (SD 3.4 spores/$m^3$) before and 4.7 spores/$m^3$ (SD 3.5 spores/$m^3$) after each chamber run.

A few sample results were excluded from analysis because sample covers were not removed (Andersen: $n = 2$), water was observed in the nozzle (Andersen: $n = 1$), or the sampling hose was observed to have been disconnected (PTFE: $n = 1$); consequently, 54 Andersen, 57 gelatin, and 56 PTFE results were available for analysis. For the gelatin results, 22 were obtained by filtering and 35 by plating analysis. All air samples were positive for CFUs with the exception of a single gelatin sample (analyzed by plating) from a low chamber run. Three samples were identified as possible outliers based on an examination of the model residuals: a PTFE sample from a low tertile run (1900 CFU/$m^3$) was approximately eight times higher than the other PTFE samples from the same run (210 and 250 CFU/$m^3$); an Andersen sample from a medium tertile run (1000 CFU/$m^3$) was low compared with the other Andersen samples from the same run (2300 and 2400 CFU/$m^3$); and a gelatin sample from the same medium tertile run (560 CFU/$m^3$) was low compared with the other gelatin samples from the same run (both 2300 CFU/$m^3$). These three outliers were influential for some analyses, so results are presented with and without outliers in the tables, but, primarily, results without outliers are primarily discussed in the text.

Mean APS estimated air concentrations for the low, medium, and high tertiles were 1600, 4100, and 9100 particles/$m^3$, respectively, with coefficients of variation (CV) ranging from 25 to 40% (Table I). Measured air concentrations were moderately correlated with the APS estimated air concentrations, with a slightly higher correlation observed for Andersen (correlation, 0.49) and PTFE (correlation, 0.49) samples compared with gelatin (correlation, 0.39) samples.

Mean measured air concentrations were not significantly different among the three sampling methods (Table I). The total CV for Andersen samples ranged from 54 to 68%, and estimated correlations among replicate Andersen samples from the same run ranged from 0.57 to 0.81. At the low tertile, Andersen and gelatin samples had lower total CVs compared with PTFE (130%) samples. At the medium and high tertiles, total CVs decreased for PTFE (79 to 62%) samples, and correlations among replicate samples of the same type increased for all sample types. When the total CV was partitioned into between- and within-run CVs, the lowest within-run CV was achieved by gelatin samplers at the highest tertile (16%), and within-run CVs were lower for the medium and high tertiles (16–35%) compared with the lowest tertile (35–65%).

Recovery efficiency (CFU/particle) did not vary by tertile (not shown), so results are presented for all tertiles combined (Table II). Statistically significant differences in mean recovery efficiency were not observed among the three air samplers ($p = 0.30$). Recovery efficiency ranged from 0.22–0.25 CFU/particle. Total CV was lower for the Andersen sampler (84%) as compared with the gelatin (97%) and PTFE (91%) filter samplers; whereas, within-run CV was lowest for gelatin filter samples (18%) compared with Andersen (25%) and PTFE (40%).

Based on the estimated recovery efficiencies, the estimated LOD for the Andersen sampler was 36 particles/$m^3$ based on a sample volume of 112 L [i.e., one CFU/(112 L $\times$ (1 $m^3$/1000 L) $\times$ 0.25 CFU/particle) = 36 particles/$m^3$], the estimated LOD for the gelatin sampler was 39 particles/$m^3$ (based on a sample volume of 120 L and a 96% sample aliquot), and for the PTFE sampler was 35 particles/$m^3$ (based on a sample volume of 120 L and a 96% sample aliquot).

## DISCUSSION

Statistically significant differences were not observed among the three air sampling methods with respect to measured concentrations ($p \geq 0.20$) or recovery efficiency ($p = 0.30$) under the conditions and methods cited.

Gelatin filters were sometimes difficult to dissolve, requiring additional plating of all the gelatin material. It is possible that the gelatin material became too dry during the 1-hr sampling period. There was one negative result observed during a low chamber run in which the other gelatin samples were 11 CFU/sample or higher (while Andersen and PTFE samples averaged 41.3 and 189.2 CFU/sample). At a minimum, sampling methods must detect the presence of *B. anthracis*. These findings make the gelatin sampling method less desirable than the other two methods. There were also three sampling results that were identified as outliers, one much higher than expected and the remaining lower. Discussions with the operator and a review of lab notebooks did not reveal any specific causes for these events.

**TABLE I. Summary of Air Sampling Results by Tertile**

| | | APS Air Concentration (particles/m$^3$) | | | | | Measured Air Concentration (CFU/m$^3$)[A] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | CV (%)[B] | | | Correlation[C] | | |
| Tertile[D] | No. Runs | Mean | CV (%)[E] | Air Sampler Type | Outliers Removed | No. Samples | Mean | $CV_B$ | $CV_W$ | $CV_T$ | A | G | P |
| Low | 6 | 1600 | 29 | Andersen | 0 | 17 | 260 | 40 | 35 | 54 | 0.57 | −0.31 | 0.51 |
| | | | | Gelatin | 0 | 18 | 160 | 54 | 37 | 65 | | 0.68 | −0.28 |
| | | | | PTFE | 1 | 16 | 460 | 113 | 65 | 130 | | | 0.75 |
| | | | | | | | p = 0.51 [F] | | | | | | |
| | | | | Andersen | 0 | 17 | 260 | 43 | 28 | 51 | 0.70 | −0.18 | 0.62 |
| | | | | Gelatin | 0 | 18 | 160 | 0 | 180 | 180 | | 0 | −0.22 |
| | | | | PTFE | 0 | 17 | 570 | 86 | 90 | 120 | | | 0.48 |
| | | | | | | | p = 0.48 | | | | | | |
| Medium | 6 | 4100 | 25 | Andersen | 1 | 17 | 1300 | 54 | 26 | 60 | 0.81 | 0.78 | 0.69 |
| | | | | Gelatin | 1 | 17 | 1300 | 57 | 17 | 60 | | 0.91 | 0.45 |
| | | | | PTFE | 0 | 18 | 1100 | 71 | 33 | 79 | | | 0.83 |
| | | | | | | | p = 0.49 | | | | | | |
| | | | | Andersen | 0 | 18 | 1200 | 44 | 37 | 58 | 0.58 | 0.60 | 0.54 |
| | | | | Gelatin | 0 | 18 | 1200 | 48 | 38 | 61 | | 0.62 | 0.25 |
| | | | | PTFE | 0 | 18 | 1100 | 72 | 29 | 78 | | | 0.86 |
| | | | | | | | p = 0.74 | | | | | | |
| High | 7 | 9100 | 40 | Andersen | 0 | 19 | 1900 | 58 | 35 | 68 | 0.73 | 0.85 | 0.76 |
| | | | | Gelatin | 0 | 21 | 1500 | 59 | 16 | 61 | | 0.93 | 0.81 |
| | | | | PTFE | 0 | 21 | 1700 | 58 | 23 | 62 | | | 0.87 |
| | | | | | | | p = 0.20 | | | | | | |

[A]To account for the correlated nature of the data, a mixed-effects regression model was used to estimate means, variances, and covariances (see text).

[B]Estimated coefficients of variation (CV) for the measured air concentrations were based on the estimated between-run (B), within-run (W), and total (T) variances from the mixed-effects regression model (see text).

[C]Estimated correlations were based on variances and covariances from the mixed-effects regression model (see text). Diagonal entries denote estimated correlations among replicate samples from the same run of the same type and off-diagonal entries denote estimated correlations among replicate samples from the same run of different types (A, Andersen; G, gelatin; and P, PTFE).

[D]Runs were classified into tertiles (low, medium, and high) based on the APS estimated air concentrations.

[E]Estimated coefficient of variation (CV) for the APS air concentrations was 100% times the sample standard deviation divided by the sample mean.

[F]P-value for the test of no differences among the measured air concentration means for the three air sampler types.

**TABLE II. Estimated Recovery Efficiency**

| No. Runs | Air Sampler Type | Outliers Removed | No. Samples | Recovery Efficiency[A] Mean | 95% CI | CV (%)[B] $CV_B$ | $CV_W$ | $CV_T$ |
|---|---|---|---|---|---|---|---|---|
| 19 | Andersen | 1 | 53 | 0.25 | 0.15–0.35 | 80 | 25 | 84 |
| | Gelatin | 1 | 56 | 0.22 | 0.12–0.32 | 95 | 18 | 97 |
| | PTFE | 1 | 55 | 0.25 | 0.15–0.35 | 82 | 40 | 91 |
| | | | | p = 0.30[C] | | | | |
| 19 | Andersen | 0 | 54 | 0.25 | 0.16–0.33 | 71 | 37 | 80 |
| | Gelatin | 0 | 57 | 0.21 | 0.12–0.30 | 83 | 43 | 94 |
| | PTFE | 0 | 56 | 0.27 | 0.16–0.39 | 71 | 78 | 110 |
| | | | | p = 0.25 | | | | |

[A]Recovery efficiency (CFU/particle) was defined as the ratio of the measured air concentration (CFU/$m^3$) and the APS estimated air concentration (particles/$m^3$). To account for the correlated nature of the data, a mixed-effects regression model was used to estimate means, variances, and covariances (see text).
[B]Estimated coefficients of variation (CV) for recovery efficiency were based on the estimated between-run (B), within-run (W), and total (T) variance from the mixed-effects regression model.
[C]P-value for the test of no differences among the recovery efficiency means for the three air sampler types.



Other studies found the Andersen sampler to be more sensitive than other air sampling methods.[3,6] Compared with this study, McCleery et al.[3] sampled much higher volumes with the Andersen sampler, which possibly accounted for the Andersen sampler being more sensitive; whereas Buttner and Stetzenbach[6] sampled similar volumes. None of the samplers tested during this study was more sensitive. In this study, only a moderate correlation between the Andersen sampler type and APS estimated concentration (0.49) was observed. Buttner and Stetzenbach reported a correlation coefficient of 0.66 for a laboratory study with *Penicillium chrysogenum* when comparing the Andersen sampler with the APS.

Juozaitis et al.[17] computed percent recovery by comparing CFUs from a culturable method (impactor or impinger) relative to the particle concentration derived from an APS using *Pseudomonas fluorescens*: 26.0% (SD 4.2) for an agar slide impactor, 24.2% (SD 4.1) for a newly developed impinger, 21.1% (SD 1.6) for Andersen VI-Stage Viable Sampler, and 9.8% (SD 2.1) for an Ace AGI-30. In our study, recovery efficiency ranged from 0.22–0.25 CFU/particle for the three samplers evaluated. Buttner and Stetzenbach[6] found the culturable percentage using the Andersen samplers to be approximately 63% relative to the APS with a size range of 1.8 to 3.5 $\mu$m for *P. chrysogenum*.

In this study, runs at the lowest concentrations did not produce many negative samples, which are needed to determine the limit of detection using probit analysis.[14] Instead, theoretical limits of detection were computed using estimated recovery efficiencies and sampling fractions using methods similar to those reported by Brown et al.[18–20] Herzog et al.[21] reviewed the literature from 1994 to 2007 on environmental LOD and found only two articles[22,23] on detection in the air. Stratis-Cullum et al.[22] used *B. subtilis* spores with an ELISA detection method and reported a LOD of 17,000 spores/$m^3$ but acknowledged that if they had analyzed the entire sample, the LOD would have been 3400 spores/$m^3$. Lester et al.[23] used *B. subtilis* spores to test an "anthrax smoke detector" and reported an LOD of 50,000 spores/$m^3$. The system detects a chemical marker of bacteria (dipicolinic acid) through fluorescence. These studies were concerned with very quick and inexpensive detection methods that resulted in high LODs.

In practice, it was difficult to create a chamber environment with a concentration as low as our initial target of 300 particles/$m^3$. Even after allowing the chamber to settle for 22 hr, the chamber did not achieve such a low level. Because of the variability of the APS at these low levels, the operator was instructed to use the prediction time to activate samplers rather than the current APS measurements. However, after all runs were completed, an error was identified in our prediction equation that resulted in activation of the samplers too early.

The "true" or APS estimated air concentration could have been determined in multiple ways. The APS sample result at the time that the samplers were activated was not used because while the number of particles counted by the APS over a 5-min period in the channels of interest was very low at the time the samplers were activated, the variability of each 5-min APS result was high. The decay prediction equation using APS data for the hours before sampler activation (in which stirred settling was occurring) was also not used because of the equation error mentioned above. The method to determine the "true" air concentration consisted of fitting two log-normal curves to the APS measurements. This method is believed to be the best estimate because it only used APS measurements during the sampling period, and it removed particles that were smaller and larger than *B. anthracis* spores.

## CONCLUSIONS

No differences were observed among the Andersen, gelatin, and PTFE air sampling methods when sampling in a chamber for *B. anthracis*. Because of difficulty in processing the gelatin filters, the gelatin samples are not preferred if either of the other two methods are available. Mean recovery efficiency ranged from 0.22 to 0.25 CFU/particle, depending on the sampling method. The estimated LOD, assuming the recovery efficiency is the same at lower concentrations, ranged from 35 to 39 particles/$m^3$. The results of this study will assist investigators in designing and implementing sampling strategies to quantify contamination levels of *B. anthracis* spores and thereby enable a more accurate risk assessment.


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