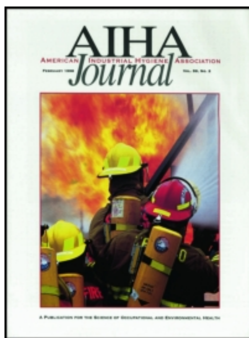

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EVALUATION OF EIGHT BIOAEROSOL SAMPLERS CHALLENGED WITH AEROSOLS OF FREE BACTERIA*

Paul Arthur Jensen[a]
William F. Todd[a]
Gregg N. Davis[b]
Pasquale V. Scarpino[c]

[a]U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Public Health Service, 4676 Columbia Parkway, Mail Stop R-5, Cincinnati, OH 45226-1099; [b]Murray State University, Department of Occupational Safety and Health, Faculty Hall, Suite 6A, Murray, KY 42071; [c]University of Cincinnati, College of Engineering, Department of Civil and Environmental Engineering, Cincinnati, OH 45221-0071

The need to quantify airborne microorganisms in the commercial microbiology industry (biotechnology) and during evaluations of indoor air quality, infectious disease outbreaks, and agriculture health investigations has shown there is a major technological void in bioaerosol sampling techniques to measure and identify viable and nonviable aerosols. As commercialization of microbiology increases and diversifies, it is increasingly necessary to assess occupational exposure to bioaerosols. Meaningful exposure estimates, by using area or environmental samplers, can only be ensured by the generation of data that are both precise and accurate. The Andersen six-stage viable (microbial) particle sizing sampler (6-STG) and the Ace Glass all-glass impinger-30 (AGI-30) have been suggested as the samplers of choice for the collection of viable microorganisms by the International Aerobiology Symposium and the American Conference of Governmental Industrial Hygienists. Some researchers consider these samplers inconvenient for evaluating industrial bioprocesses and indoor or outdoor environments. Alternative samplers for the collection of bioaerosols are available; however, limited information has been reported on their collection efficiencies. A study of the relative sampling efficiencies of eight bioaerosol samplers has been completed. Eight samplers were individually challenged with a bioaerosol, created with a Collison nebulizer, of either Bacillus subtilis *or* Escherichia coli. *The samplers were evaluated under controlled conditions in a horizontal bioaerosol chamber. During each experimental run, simultaneous samples were collected with a reference AGI-30 to verify the concentration of microorganisms in the chamber from run to run and day to day. The results of this research indicate a wide variation in sample collection efficiency for free bacteria (i.e., mostly single cells of* E. coli *and* B. subtilis, *$d_{ac} \leq 2$ µm). The particle concentration in the aerosol chamber, as indicated by the AGI-30 sampler, was 2000 ± 85 colony-forming units per cubic meter (CFU/m^3) for* E. coli *and 1170 ± 400 CFU/m^3 for* B. subtilis. *The collection efficiency of the evaluated AGI-30 relative to the reference AGI-30 was 101% ± 4%. The 6-STG oversampled the reference AGI-30 by approximately 7%. However, the Andersen single-stage (1-STG), Mattson-Garvin Slit-to-Agar (STA), and Andersen two-stage (2-STG) samplers undersampled the reference AGI-30 by 7%, 10%, and 33%, respectively. The relative collection efficiencies of the Gelman 47-mm membrane filter (MF), Pool Bioanalysis Italiana surface air system (SAS), and Biotest Reuter centrifugal sampler (RCS) were <1% for* E. coli. *The low relative efficiency of the MF with* E. coli *was likely caused by desiccation of the organism. The SAS and RCS samplers, because of their design, are not efficient collectors of small particles. The relative efficiency of the MF with* B. subtilis *was 3% lower than that of the reference AGI-30 because this organism is an endospore-former and is more resistant to desiccation. For aerosols of free bacteria, the Andersen six-stage impactor, the Ace Glass AGI-30, and the Andersen single-stage impactor gave comparable results.*

Currently, occupational health investigators are assessing worker exposure to microorganisms by using various air samplers designed for monitoring viable microbial aerosols. However, these currently available air samplers cannot recover viable airborne particles without some inactivation or loss during or after sampling. Consequently, the effectiveness of air monitoring depends on the sampler used and the nature of the aerosol sampled.

*Disclaimer: Mention of commercial names or products does not constitute endorsement by the National Institute for Occupational Safety and Health. This work was supported, in part, by the U.S. Environmental Protection Agency, Office of Research and Development, through Interagency Agreement DW75932393-01-2.

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The Andersen (Atlanta, Ga.) six-stage viable (microbial) particle sizing sampler (6-STG) and the Ace Glass (Vineland, N.J.) all-glass impinger-30 (AGI-30) have been regarded as the samplers of choice for the enumeration of viable microorganisms.[1–3] Several studies have compared currently available bioaerosol samplers.[4–10] However, the majority of these comparison studies are based on side-by-side sampling conducted under field conditions. Because of the inability to control extraneous variables in field studies, many of the results may have been influenced by the variability of the microorganisms sampled (viability issues), the general size distribution of the aerosols sampled, and the continuously changing conditions of the ambient environment. Because of limited documentation of environmentally controlled sampler evaluations, a study was initiated to evaluate several bioaerosol samplers in an aerosol chamber under controlled experimental conditions. The samplers were challenged with an aerosolized suspension of either *Escherichia coli* or *Bacillus subtilis*. This laboratory evaluation eliminated much of the variability encountered in field studies.

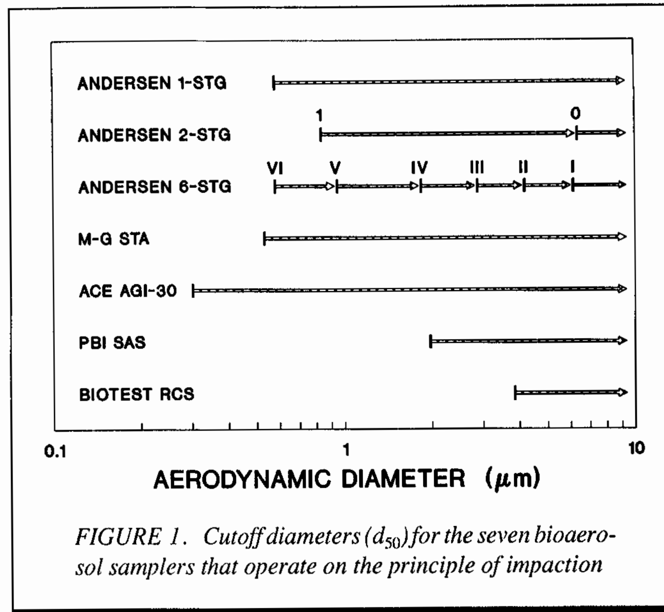

FIGURE 1. Cutoff diameters (d_{50}) for the seven bioaerosol samplers that operate on the principle of impaction

EXPERIMENTAL MATERIALS AND METHODS

Aerosol Samplers

The following samplers were evaluated. Abbreviations used in this paper are shown in parentheses.

- Andersen six-stage viable (microbial) particle sizing sampler (6-STG, Andersen Instruments Incorporated, Atlanta, Ga.)
- Andersen two-stage viable (microbial) particle sizing sampler (2-STG)
- Andersen single-stage viable (microbial) particle sampler (1-STG)
- Pool Bioanalysis Italiana (PBI) surface air system sampler (SAS, Pool Bioanalysis Italiana, Milano, Italy; Spiral System Instruments, Bethesda, Md.)
- Mattson-Garvin Slit-to-Agar air sampler (STA, Barramundi Corporation, formerly Mattson-Garvin Company, Homosassa Springs, Fla.)
- Biotest Reuter centrifugal air sampler (RCS, Biotest-Serum-Institut GmbH, Frankfurt, Germany; Biotest Diagnostics Corporation, Denville, N.J.)
- Gelman 47-mm membrane filter air sampler (MF, Gelman Sciences, Ann Arbor, Mich.)
- Ace Glass all-glass impinger-30 sampler (AGI-30, Ace Glass, Vineland, N.J.)

Aerosol Sampler Operation

Andersen Six-Stage Viable (Microbial) Particle Sizing Sampler

The Andersen 6-STG sampler is a multi-orifice, cascade impactor with 400 holes per stage, drawing air at a flow rate of 28.3 L/min. Particle velocity increases as the air flows through successively smaller holes. Large particles, ≥7 µm, impact on the first stage and smaller particles follow until accelerated sufficiently to impact at a later stage. This sampler was designed so that all particles collected, regardless of physical size, shape, or density, are sized aerodynamically and can be directly related to human lung deposition.[11]

As a practical matter, an impactor such as this can be assumed to be ideal and the efficiency curves characterized by a single number, Stk_{50}, the Stokes number that gives 50% collection efficiency. This is equivalent to assuming that the mass of the particles larger and smaller than the cut-diameter (d_{50}) are equal. Hence, the d_{50} is the aerodynamic diameter above which the collection efficiency of the impactor approaches 100%.[12] Aerodynamic diameter (d_{ae}) is the diameter of a unit density ($\rho_p = 1$ g/cm^3) sphere that has the same settling velocity as the particle. Figure 1 shows the d_{50} for Stages 1–6 of the Andersen 6-STG sampler to be 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 µm, respectively.[11]

Sampling plates were prepared by pouring 45 mL of Bacto tryptic soy agar (TSA, DIFCO Laboratories, Detroit, Mich.) aseptically into each of the six 100-mm × 15-mm sterile plastic petri plates so that the gap between the nozzles and agar surface met the manufacturer's specification.[13,14] All inside surfaces were maintained in a sterile condition until sampling. After sampling for approximately 180 sec or the equivalent of 84.9 L, the plates were removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 hr. The CFU on all six plates were combined for analysis.

Andersen Two-Stage Viable (Microbial) Particle Sizing Sampler

The Andersen 2-STG sampler is also a multi-orifice, cascade impactor with 200 holes per stage, drawing air at a flow rate of 28.3 L/min. Figure 1 shows the d_{50} for Stages 0 and 1 to be 8.0 and 0.95 µm, respectively.[13] The two sampling plates were each prepared aseptically with 20 mL of TSA so that the gap between the nozzles and agar surface met the manufacturer's specification.[13] After sampling for approximately 180 sec or the equivalent of 84.9 L, the plates were removed from the sampler and handled as described above. The CFU on both plates were combined for analysis.

Andersen Single-Stage Viable (Microbial) Particle Sampler

The Andersen 1-STG sampler is the sixth stage of the Andersen six-stage sampler, drawing air at a flow rate of 28.3 L/min. Figure 1 shows the d_{50} of the sixth stage of the sampler as 0.65 µm.[11] The plate was prepared and handled as described above for the 6-STG sampler.

Pool Bioanalysis Italiana Surface Air System Sampler

The PBI SAS samplers operate similarly in principle to the Andersen 1-STG sampler. The PBI SAS compact sampler was used for this study, and the impactor stage consists of 219 holes. The holes are approximately the same diameter as the second stage of the Andersen 6-STG sampler, but because the sampling rate is 90 L/min, the d_{50} is approximately 2 µm.[8] The SAS sampler has an internal timer and can be set to sample from 20 sec to 5 min in 20-sec increments. A 65-mm × 15-mm replicate organism detection and counting (RODAC®, Becton Dickinson, Cockeysville, Md.) plate was prepared aseptically with approximately 16 mL TSA so that a convex meniscus formed at the rim of the plate.[8] After sampling for approximately 300 sec or the equivalent of 450 L, the plate was handled as described above.

The other PBI SAS model operates at a flow rate of 180 L/min. This model has an impactor stage with 260 holes and can use a 90-mm RODAC plate.[8] This sampler was not used in this evaluation.

Mattson-Garvin Slit-to-Agar Air Sampler

The STA sampler uses inertial impaction to collect organisms onto a culture medium at a flow rate of 28.3 L/min. The sampler has a tapered slit that produces a rectangular jet stream toward the surface of a rotating petri plate when air is sampled. Figure 1 shows the calculated d_{50} of the STA sampler as approximately 0.5 µm. A 150-mm × 15-mm plastic petri plate was prepared aseptically with 60 mL of TSA for each so that the gap between the sieve and agar surface met the manufacturer's specification.[15] After sampling for approximately 180 sec or the equivalent of 84.9 L, the plates were handled as described above.

Biotest Reuter Centrifugal Air Sampler

The RCS sampler also uses inertial impaction to collect viable organisms onto a culture medium. The manufacturer states that the measured flow rate (termed *sampling volume* by Biotest) of the sampler is 280 L/min; however, the flow rate was experimentally determined to be approximately 210 L/min by Macher and First.[6,7,16,17] Because the air enters and exits the same opening, the actual volumetric flow rate is not easy to evaluate. Air was impacted onto a commercially available plastic strip containing 34 wells of TSA (Biotest). Each well had an area of approximately 1 cm^2. Figure 1 shows the d_{50} of the RCS sampler as approximately 4 µm, which was determined from the experimental data of Macher and First.[17] The manufacturer recommends using a flow rate of 40 L/min (termed *separation volume* by Biotest) for a particle diameter of 4 µm. In other words, the manufacturer is stating that the sampler is 100% efficient at collecting 4-µm particles, rather than stating that it is only 14% efficient, on a theoretical basis. If a flow rate of 40 L/min is used in lieu of the actual or measured flow rate, then the concentration of particles less than 4 µm will be underestimated and the percentage of particles greater than 4 µm will be overestimated. After sampling for approximately 480 sec or the equivalent of 1680–2240 L, the strip was removed from the sampler and handled as described above. The manufacturer's flow rate of 280 L/min was used for calculating the concentration of bacteria in air.

Gelman 47-mm Membrane Filter Air Sampler

The MF sampler is widely used for aerosol sampling because of its low cost and simplicity of operation. However, most vegetative bacteria are too fragile for collection by this method.[18] This air filtration apparatus consists of an aluminum, 47-mm, open-faced filter holder with a sterile, 47-mm membrane filter made of cellulose esters, with a 0.45-µm pore size (GN-6, Gelman), mounted with a sterile backup pad and connected to a vacuum source through a flow rate controller (i.e., critical orifice).[19] After sampling for approximately 480 sec at a flow rate of 5 L/min, or the equivalent of 40 L, the membrane filter was removed from the sampler. The membrane filter was placed in a 50-mm petri plate on a sterile pad saturated with Bacto tryptic soy broth (TSB, DIFCO). The plate with the MF and pad was covered and handled as described above.

Ace Glass All-Glass Impinger-30 Sampler

The AGI-30 sampler is a high-velocity liquid impinger widely used for air sample collection. Figure 1 shows the d_{50} of the AGI-30 sampler as approximately 0.3 µm.[20] The AGI-30 sampler operates by drawing aerosols at a flow rate of 12.3–12.6 L/min through an inlet tube curved to simulate the nasal passage.[21] In contrast to impaction of bioaerosols onto agar, impingers may give higher bacterial counts in environments where bacteria are carried as aggregates, mainly because bacterial clusters are broken up.[22] After sampling for approximately 360 sec or the equivalent of 75 L, quantifying of airborne microbes was accomplished by using a membrane filtration plating technique because the expected microbial load was low.[23] The membrane filter was placed in a 50-mm petri plate on a pad saturated with TSB. The plate with the MF and pad was handled as described above.

Sampler Flow Rate Calibration

The volumetric flow rate of air through the 6-STG, 2-STG, 1-STG, AGI-30, MF, and STA samplers were calibrated to within the manufacturers' specifications using a primary standard (spirometer).[24] The flow rate through these sampling instruments was verified to be within manufacturers' specifications before each day of sampling by using a dry gas meter (Parkinson Cowan Industrial Products, London, England) that had been previously calibrated against the same primary standard. Because the fan in the SAS sampler is not powerful enough to overcome the pressure drop across the dry gas meter, the flow rate was verified by exhausting a measured volume (90 L) of air from a plastic bag through the SAS. The sampling rate was found to be within 10% of the manufacturer's specification (90 L/min). The RCS is not easy to calibrate and the flow rate is difficult to verify.[17] The authors followed the manufacturer's recommendation by using

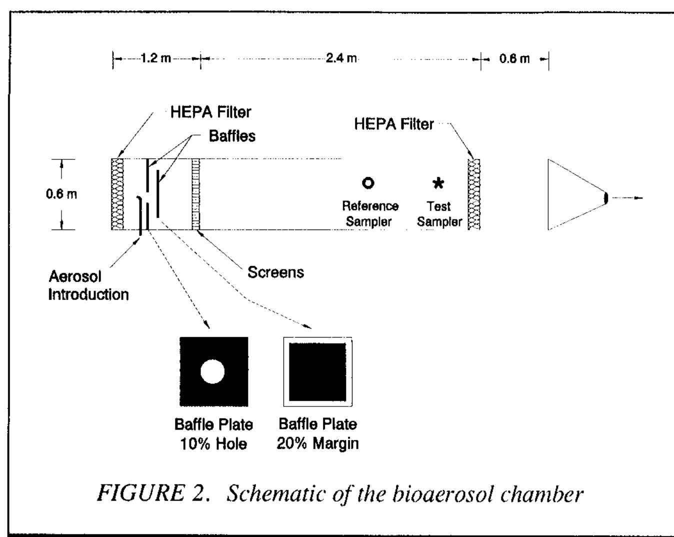

FIGURE 2. Schematic of the bioaerosol chamber

the Calibration-Set (Biotest) to check and, if necessary, correct the impeller blade angle. In addition, the internal timer was verified to within the manufacturer's specification (8 min ± 2%) with a stopwatch.

Aerosol Chamber

The aerosol chamber used in this study was similar in design to the Air Cleaner Standard Test Duct recommended by the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) for rating air cleaners.[25] The chamber was a stainless steel 0.61-m square duct, approximately 3.7 m long. Two baffles were located downstream of the aerosol inlet to promote uniform mixing of the aerosol with the airstream. The chamber supply and exhaust air was purified with high-efficiency particulate air (HEPA) filters. In addition, the HEPA-filtered chamber exhaust air was ducted to the roof of the laboratory through an existing stack. Four side hatches allowed easy access to the inside of the chamber to place and remove samplers and allowed application of chemical disinfectants. A schematic diagram of this aerosol chamber is shown in Figure 2. Temperature and relative humidity (RH) to the laboratory was controlled, and these parameters were measured in the aerosol chamber to range from 21 to 24°C and 45% to 55% RH.

The flow rate of air through the chamber was adjustable, but for the purpose of this study, the flow rate was maintained at 11.3 m^3/min (11 300 L/min). Vertical and horizontal velocity traverses were performed by using a hot-wire anemometer. The velocity throughout the cross-sectional area of the sampler location was uniform and determined to be 0.54 m/sec ± 0.014 m/sec. To verify that the aerosol was uniformly distributed across the same cross-sectional area, a monodisperse aerosol of oleic acid was generated by using a Berglund-Liu vibrating orifice monodisperse aerosol generator.[26,27] An aerosol with a d_{ae} of approximately 5 μm entered the chamber through the aerosol generation port. Both the size of the particles generated and the mass concentration of the particles generated were uniform across the cross-sectional area where the samplers were located. The mass median aerodynamic diameter for the horizontal and vertical traverses was measured by using a TSI aerodynamic particle sizer (APS, TSI Inc., St. Paul, Minn.) and found to be 5.37 μm with a geometric standard deviation (GSD) of 1.13. As defined by Hinds,[12] a monodisperse aerosol is one that has a GSD of less than 1.2. The mass concentration for these traverses was measured to be 0.0136 mg/m^3 ± 0.0016 mg/m^3.

Preparation of Pure Culture Suspensions

Cultures used in this study included *E. coli* (American Type Culture Collection (ATCC) 11229) and *B. subtilis* (ATCC 6633, DIFCO). *E. coli* is a gram-negative, nonspore-forming bacterium that is approximately 0.5 μm wide and 2.0 μm long. *B. subtilis* is a gram-positive, endospore-forming bacterium of similar size and shape. The endospores of *B. subtilis* were less than 0.5 μm in diameter. Active *E. coli* and *B. subtilis* cultures were inoculated into approximately 100 mL of TSB and incubated for 18–24 hr at 35°C in Erlenmeyer flasks in a shaker-incubator. The broth was then aseptically transferred to a 175-mL sterile conical centrifuge tube (Falcon® 2076, Becton Dickinson and Company, Lincoln Park, N.J.), capped, and centrifuged at 1500 G for 10 min in an IEC CENTRA®-4B general-purpose centrifuge (International Equipment Co., Needham, Mass.). The supernatant was discarded and the pellet was resuspended in sterile phosphate-buffered dilution water. This washing process was repeated two more times and the cells were resuspended a fourth time in approximately 100 mL of sterile phosphate-buffered dilution water. Microscopic examination of the suspension of *E. coli* showed motile, single cells with a minute amount of cell debris. Microscopic examination of the suspension of *B. subtilis* showed motile, single cells with an endospore intact and a minute amount of cell debris. The cell concentration was determined by turbidimetric measurement of this suspension. The transmittance of monochromatic light at a wavelength of 450 nm through the cell suspension was measured by using a Spectronic® 20 spectrophotometer (Milton Roy Co., formerly Bausch & Lomb, Rochester, N.Y.). The concentration of cells was estimated from previously developed calibration curves for the spectrophotometer, and the appropriate dilution was made to attain a total volume of 100 mL with a concentration of approximately 10^6 bacterial cells per milliliter solution for use in the aerosol generator.[28] A dilution series was performed for standard plate count enumeration.[29] The nebulizer then generated an aerosol of predominately single bacterial cells with a chamber concentration of approximately 2000 CFU/m^3 of *E. coli* or 1200 CFU/m^3 of *B. subtilis*.

Aerosol Generation

A Collison nebulizer was used to aerosolize the suspensions of *E. coli* cells and *B. subtilis* cells. The BGI six-jet modified MRE-type Collison Nebulizer (BGI Collison Nebulizer, BGI Inc., Waltham, Mass.) was used in this study. Within the nebulizer, compressed air expanded from a pressure of 140 kPa at the stem into six side jets. The reduction of static pressure forced water up the tube at the bottom of the stem, similar to an eductor. An eductor is an ejector-like device for mixing two fluids (e.g., a liquid and a gas). This liquid suspension was then broken up by the air jet into a dispersion of droplets of very wide size distribution. Most of the droplets were blown onto the internal

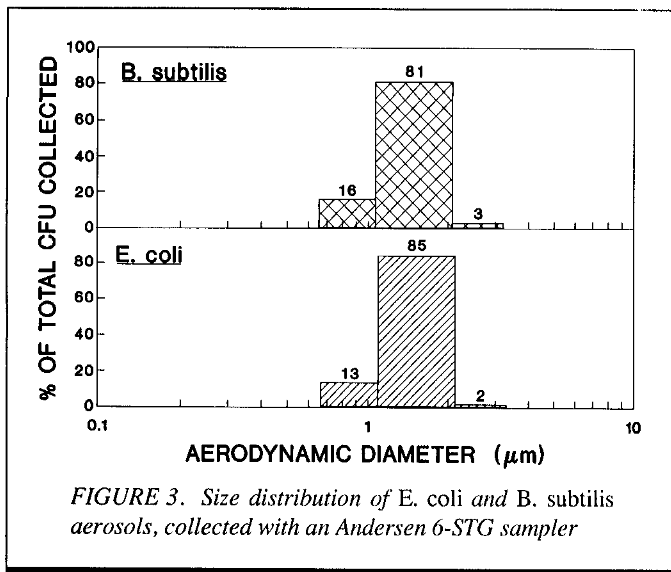

FIGURE 3. Size distribution of E. coli *and* B. subtilis *aerosols, collected with an Andersen 6-STG sampler*

wall of the glass vessel; however, the minute amount of liquid that escaped impaction comprised only the finest tail of the droplet-size distribution and these droplets were carried up and out of the nebulizer by the spent air. Because the droplets in this emerging air were aqueous, they evaporated very rapidly on admixture with unsaturated air. For example, a 10-μm water droplet in air at 20°C and 80% RH would have had a wet lifetime of 0.6 sec; whereas a 2.9-μm water droplet would have had a wet lifetime of 0.03 sec. The wet lifetime is proportional to diameter squared at a given temperature and relative humidity.[30] Published results of aerosol distributions indicate that the BGI Collison nebulizer generates droplets of mass median diameter of 2.9 μm with a geometric standard deviation of 3.17.[31] Theoretically, predominantly single bacterial cells made their way to the sampler location in the aerosol chamber, a transit time of approximately 6 sec or approximately 10 lifetimes for a 10-μm water droplet. To validate the assumption that the generated particles contained either one bacterium and water, or just water, a clean glass slide was placed within 5 cm of the aerosol outlet of the Collison nebulizer to collect a sample of the aerosol generated. The slide was viewed by using a phase contrast microscope (Labophot-2, Nikon Corp., Tokyo, Japan). No clumping of bacteria was observed. Also, some cell debris, including a few endospores of *B. subtilis*, were observed.

The distributions of CFUs collected on the Andersen 6-STG sampler for both *E. coli* and *B. subtilis* are shown in Figure 3. The distributions indicate that a majority of the particles (≥97%) collected had a d_{ae} less than or equal to 2.1 μm. Aerosols of free bacteria, such as those generated in this study, are commonly found in home, sugar beet processing, and machining environments.[32–34] In fact, in the normal environment, the particle size distribution of viable bacteria particles is quite wide with a mode between 3 and 5 μm.[35,36]

The viability of *E. coli* and *B. subtilis* in the nebulizer was tested independently. A 100-mL suspension of approximately 10^6 cells/mL phosphate-buffered dilution water was placed both in a sterile nebulizer jar and in a sterile Collison nebulizer. The nebulizer was cycled (5 min on and 5 min off for 90 min, and then left static for 210 min) and aliquots of the nebulized solution were diluted and plated. In parallel, aliquots of the suspension (static cells) were also diluted and plated over the same 300-min period. The death rate of the nebulized cells was similar to the death rate of the static cells. Furthermore, in either case, the reduction in viability was less than one-tenth of an order of magnitude after 5 hr, indicating that *E. coli* and *B. subtilis* remain viable in phosphate-buffered dilution water for extended periods of time.

Enumeration of Bacteria

After incubation for 24 hr at a temperature of 35°C in a Forma Scientific water-jacketed incubator Model 3158 (Forma Scientific, Marietta, Ohio), all plates were enumerated by using a New Brunswick Model C-110W colony counter (New Brunswick Scientific Co., Edison, N.J.). All CFUs on each plate were counted, including a number of "multiple hits." Because it was very easy to identify more than one CFU at an impaction point, a positive hole statistical adjustment was not applied. Detailed discussions of the positive hole statistical adjustment are found in the literature.[11,37,38]

Experimental Design

The eight bioaerosol samplers were compared to an AGI-30 sampler located approximately 0.6 m upstream of the test sampler location. This AGI-30 was considered the reference condition because these samples were taken in parallel with all the other samplers and used to normalize the data for any variance of microbial aerosol concentration from run-to-run or day-to-day. The AGI-30 was chosen as the reference sampler because of its well-documented acceptance and use in the collection of bioaerosols and because it has a calculated d_{50} of 0.30 μm. All samplers were kept aseptic by covering with sterile aluminum foil. Prior to placing the reference AGI-30 and one test sampler in the chamber, the foil was removed. The samplers were placed in the chamber in their "natural" orientation. The 6-STG, 2-STG, 1-STG, and STA samplers were placed such that the inlet was normal to the airflow. The inlets of the SAS, RCS, AGI-30, and MF were oriented into the airflow. May and Druett[39] showed that particles with a d_{ae} less than 7.5 μm are not affected greatly by anisokinetic sampling conditions such as those used in this study. In conditions of air velocities greater than 2 m/sec, isokinetic sampling is required.[3] The chamber fan was activated and the air supply to the nebulizer was adjusted to 140 kPa. After waiting 1 min for the aerosol concentration to reach equilibrium, the sampling pumps were turned on. After the appropriate sampling time, the sampling pumps and the air supply to the nebulizer were turned off. Again, the chamber was allowed to purge aerosolized bacteria for 1 min. The samplers were removed from the chamber and aseptically handled as previously described. Each of the eight tested samplers were evaluated on 5 separate days. During each of the 10 sampling sessions (one session per day), four different samplers were evaluated four times each. The order in which the samplers were tested was randomized, resulting in an incomplete randomized block design.

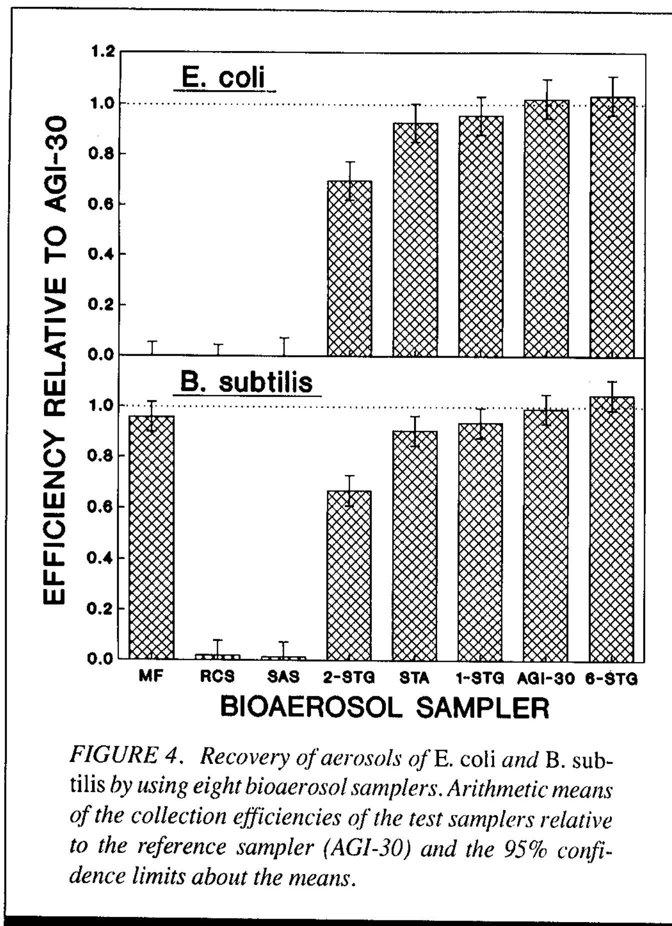

FIGURE 4. Recovery of aerosols of E. coli *and* B. subtilis *by using eight bioaerosol samplers. Arithmetic means of the collection efficiencies of the test samplers relative to the reference sampler (AGI-30) and the 95% confidence limits about the means.*

Statistical Analysis

All statistical analyses were performed by using SAS Version 6.03.[40] To normalize for any variation in bacteria concentration in the chamber (i.e., variation from run-to-run, within each day, and from day-to-day), analyses were performed on the ratio of the concentration of bacteria collected by the test sampler to the concentration of bacteria collected by the reference sampler (i.e., AGI-30). This ratio will be referred to as the collection efficiency relative to the AGI-30. Analysis of variance (ANOVA) tables were generated by using the general linear models procedure (PROC GLM). The dependent variable, collection efficiency relative to the AGI-30, was regressed with three independent variables: sampler type (e.g., 1-STG, 2-STG, 6-STG, STA, RCS, SAS, MF, or AGI-30); day of evaluation; and sequence within each day.

RESULTS

Overall Evaluation of Eight Bioaerosol Samplers

For each sampler type, the collection efficiency relative to the AGI-30 was not significantly different by day or sequence within each day, indicating that the ratio is a valid method to normalize the data. The same test was performed on the concentration of bacteria collected by the reference sampler (AGI-30). The concentration of bacterial aerosol did not change significantly during any one day, but did vary somewhat from day to day. Because of this result, the means of the four replicate concentration efficiencies relative to the AGI-30 were used for any further analysis. The means and the 95% confidence intervals for the concentration efficiencies relative to the AGI-30 are shown in Figure 4. The means, the 95% confidence intervals, and the results of the multiple comparison tests of the dependent variable (collection efficiency relative to the AGI-30) for each sampler type are shown in Table I. Because all comparisons were of interest, the Bonferroni t statistics as computed by Dunn were utilized. For *E. coli*, the relative collection efficiency of the following combinations of samplers were not significantly different: 6-STG and AGI-30; AGI-30 and 1-STG; 1-STG and STA; and SAS, RCS, and MF. For *B. subtilis*, the relative collection efficiency of the following combinations of samplers were not significantly different: 6-STG and AGI-30; AGI-30, MF, and 1-STG; MF, 1-STG, and STA; and SAS and RCS.

TABLE I. Bonferroni (Dunn) t Tests and Means ±95% Confidence Limits about the Means of the Dependent Variable, Collection Efficiency Relative to the AGI-30 for Samplers Challenged with Aerosols of *E. coli* and *B. subtilis*

	E. coli		B. subtilis	
Sampler	*Bonferroni*	*Mean ± CLM*	*Bonferroni*	*Mean ± CLM*
6-STG	A	1.071 ± 0.065	F	1.059 ± 0.063
AGI-30	B A	1.001 ± 0.036	G F	1.014 ± 0.043
1-STG	B C	0.934 ± 0.087	G H	0.926 ± 0.044
STA	C	0.887 ± 0.078	H	0.907 ± 0.055
2-STG	D	0.681 ± 0.062	I	0.667 ± 0.033
SAS	E	0.00089 ± 0.00096	J	0.00263 ± 0.01
RCS	E	0.00028 ± 0.00015	J	0.00015 ± 0.00016
MF	E	<0.00001 ± <0.0000002	G H	0.966 ± 0.048

Note: Means with the same letter are not significantly different (α = 0.003125).

Recovery of Vegetative Cells versus Bacterial Endospore-Formers

Data shown in Figure 4 compare the recovery of free bacteria aerosols of vegetative cells (*E. coli*) and bacterial endospore-formers (*B. subtilis*). The major difference between the vegetative cells and the bacterial endospore-formers was observed in the performance of the MF sampler. The low relative efficiency of the MF with *E. coli* was likely because of its desiccation on the membrane filter. *B. subtilis* is an endospore-former and is more resistant to desiccation, thus resulting in a MF relative collection efficiency similar to that of the reference AGI-30.

DISCUSSION AND CONCLUSIONS

The equivalent relative collection efficiencies of the Andersen 6-STG sampler, Andersen 1-STG, and Ace Glass AGI-30 in recovering free bacteria confirm other reports in the literature.[4,5,41-43] Usually, impaction methods give higher particle

recovery than other methods. Impaction onto the nutrient medium such as agar located a few millimeters below the perforated plate results in low sampling stresses and post-collection sample manipulation is not required.

Viability loss may occur in the AGI-30 because of the amount of shear force involved in collection. The airstream approaches sonic velocity, resulting in almost complete collection of suspended particles by impingement in the liquid collection medium. This condition tends to cause the destruction of some vegetative cells or may result in overestimation because of the dispersion of dust particles and the breaking up of clumps of bacteria.[20,21,42–44]

The Mattson-Garvin STA relative sampling efficiency was marginally lower than the 6-STG, 1-STG, AGI-30, and MF for *B. subtilis*. Because of the low d_{50} (0.5 μm), the STA should have a relative sampling efficiency similar to the 6-STG, 1-STG, and AGI-30 and supports other comparisons.[45–47] However, the RCS was found to be more efficient than the STA in one study and less efficient in another.[9,47]

The relative efficiency of the Andersen 2-STG sampler for this particular particle size distribution was significantly lower than the other two Andersen samplers, the AGI-30, and the STA.[4] By superimposing Figure 1 on Figure 3, it is clear that the lower tail of the aerosol distribution is below the d_{50} of Stage 1 (the lower stage) of the 2-STG, thus explaining the lower relative efficiency.

The PBI SAS and Biotest RCS samplers collected very few microorganisms. The collection efficiency of these samplers is dependent on the particle size of the aerosol being sampled. For the RCS, both the experimental collection efficiency for small particles (<2 μm) determined by Macher and First[17] and the theoretical efficiency for small particles calculated by using the empirical formula developed by Biotest indicate the poor collection characteristics of this instrument.[16] With a d_{50} of 4 μm for the RCS and a d_{50} of 2 μm for the SAS, few if any free bacteria would impact on the agar surface. The advantage of portability of these two instruments could, in part, compensate their inefficiency of collecting small particles such as free bacteria if the bioaerosol is well characterized and the d_{ae} is greater than 4 or 2 μm, respectively. These samplers have shown significantly better recovery of larger particles.[7,8,17]

The vegetative cells of *E. coli* were essentially killed by desiccation with the Gelman MF sampler; the endospore-forming, desiccation-resistant cells of *B. subtilis* resulted in relative collection efficiencies similar to those of the Andersen 6-STG, Andersen 1-STG, and Ace Glass AGI-30 samplers.[10,45,48,49] The MF sampler is widely used for aerosol sampling because of its low cost and simplicity of operation; but most vegetative bacteria are too fragile for collection by this method.[18]

The results of this study indicate that the Andersen 6-STG, Andersen 1-STG, and Ace Glass AGI-30 samplers were the samplers of choice for recovering aerosols of free bacteria (i.e., mostly single cells of *E. coli* and *B. subtilis*) under the conditions of this study. The Ace Glass AGI-30 and the Andersen 6-STG have been suggested as the samplers of choice for the enumeration of viable microorganisms.[1–3] The Andersen 1-STG had been suggested by the American Conference of Governmental Industrial Hygienists Committee on Bioaerosols (ACGIH COB) as a sampler of choice; however, the ACGIH COB no longer recommends any one sampler as the sampler of choice.[18,50] The comparison of these bioaerosol samplers may not be valid under conditions different than those imposed for this study; thus, the equivalency of collection efficiencies for all bioaerosol samplers must be determined for each environmental condition in which they are to be used. As previously described, aerosols of free bacteria or bacteria clumped with small particles can be found in both indoor and outdoor environments. If the conditions of sampling cannot be determined, then the 6-STG, 1-STG, or AGI-30 should be used to ensure that an air sample representative of the entire particle size distribution of bioaerosols is collected.

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