EVALUATION OF SAMPLING ALTERNATIVES FOR BIOAEROSOLS

PHASE II: VIABLE MICROORGANISM SAMPLING EFFICIENCY

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16. Abstract (Limit: 200 words) A study was undertaken of the relative sampling efficiencies of eight bioaerosol samples. Each was individually challenged with a bioaerosol, created with a Collision nebulizer, of either Bacillus-subtilis or Escherichia-coli. The samplers were evaluated under controlled conditions in a horizontal bioaerosol chamber. During each run simultaneous samples were collected with a reference Ace Glass All Glass Impinger-30 (AGI-30) to verify the concentration of microorganisms in the chamber from run to run and day to day. The results indicated a wide variation in sample collection efficiency for free bacteria. The collection efficiency of the evaluated AGI-30 relative to the reference AGI-30 was 100%. The Andersen Six Stage Viable Particle Sizing Sampler (6-STG) over sampled the reference AGI-30 by about 7%. However, the Andersen Two Stage samplers undersampled the reference AGI-30 by 8%, 11%, and 32%, respectively. The relative collection efficiencies of the Gelman 47mm Membrane Filter, PBI Surface Air System and Biotest Reuter Centrifugal Sampler were less than 1% for E-coli. The low relative efficiency of the Membrane Filter with E-coli was probably due to desiccation of the organism. The Surface Air System and the Reuter Centrifugal Sampler were not efficient collectors of small particles. The relative efficiency of the Membrane Filter with B-subtilis was similar to that of the reference AGI-30. For aerosols of free bacteria, the Andersen Six Stage impactor, the Ace Glass AGI-30, and the Andersen One Stage impactor gave comparable results.

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ABSTRACT

The need to quantify airborne microorganisms in the commercial microbiology industry (biotechnology) and during evaluations of indoor air quality (IAQ), 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 non-viable aerosols. As commercialization of microbiology increases and diversifies, it is increasingly necessary to accurately assess occupational exposure to bioaerosols. Exposure estimates, using area or environmental samplers, can only be assured by the generation of data that are both precise and accurate. The Andersen Six-stage Viable 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 ACGIH. 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, $d_{ae} \leq 2$ μm). The particle concentration in the aerosol chamber as indicated by the AGI-30 sampler was 2000 ± 270 Colony Forming Units per cubic meter (CFU/m^3). The collection efficiency of the evaluated AGI-30 relative to the reference AGI-30 was 100% ± 4%. The 6-STG over-sampled 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 under-sampled the reference AGI-30 by 8%, 11%, and 32% respectively. The relative collection efficiencies of the Gelman 47 mm Membrane Filter (MF), PBI 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 due to 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 similar to that of the reference AGI-30, because the organism is an endospore-former and is more resistant to desiccation. For aerosols of free bacteria, the Andersen 6-stage impactor, the Ace Glass AGI-30, and the Andersen 1-stage impactor gave comparable results.

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The authors wish to thank Pasquale V. Scarpino, Ph.D., Professor of Environmental Engineering (Microbiology) and Environmental Health, Department of Civil and Environmental Engineering, College of Engineering, University of Cincinnati for his review of laboratory technique, experimental design, sampling methods, enumeration methods, and quality control/quality assurance used in this project. Dr. Scarpino also provided the stock cultures to our laboratory. The authors also thank Ronald M. Hall, Physical Science Technician, Engineering Control Technology Branch, Division of Physical Sciences and Engineering, National Institute for Occupational Safety and Health for his excellent drawings of bioaerosol samplers and equipment.

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I. INTRODUCTION

The need to quantify airborne microorganisms in the commercial microbiolog industry (biotechnology) and during evaluations of indoor air quality (IA infectious disease outbreaks, and agriculture health investigations has own there is a major technological void in bioaerosol sampling techniques t measure and identify viable and non-viable aerosols. As the commercialization of microbiology increases and diversifies, it is increasingly necessary to accurately assess occupational exposure to bioaerosols. Exposure estimates, using area or environmental samplers, can only be assured by the generation of data that are both precise and accurate. Both the National Institute for Occupational Safety and Health (NIOSH), Division of Physical Sciences and Engineering, Engineering Control Technology Branch, and the United States Environmental Protection Agency (EPA), Office of Research and Development, Risk Reduction Engineering Laboratory, have interests in the efficacy of the containment of processes which use genetically engineered microorganisms and entered into an interagency agreement (IA) to conduct research in this area.

NIOSH has published a control technology assessment of enzyme fermentation processes.[1] Risk assessment techniques based on the data from this field work will be applied to the biotechnology industry by EPA. The first step in this IA was to develop improved sampling techniques for field work, and use these techniques to generate additional data. In the first phase of this IA, an evaluation of five bioaerosol samplers was performed using a chemical surrogate in lieu of a viable microorganism to gain an appreciation for the relative physical efficiency of the samplers when challenged with aerosols of

three discrete particle diameters. The results are discussed in an interim report submitted to EPA.[2]

Recommended samplers for viable microorganisms and their basic principles of operation are discussed in the literature and in the interim report to EPA, and will not be repeated.[2] The Andersen Six-Stage Viable (Microbial) Particle Sizing Sampler and the Ace Glass All Glass Impinger-30 have been regarded as samplers of choice for the enumeration of viable microorganisms.[3] In this second phase of this IA, eight microbial samplers were challenged with a viable microorganism and compared to a reference sampler upstream of the test samplers. The methods and results of the second phase of the IA are set forth in this report.

II. METHODS AND MATERIALS

A. Aerosol Samplers

The following samplers were evaluated:

- 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 Bionalyse Italiana (PBI) Surface Air System Sampler (SAS, Pool Bionalyse Italiana, Milano, Italy);

- Mattson-Garvin Slit-to-Agar Air Sampler (STA, Barramundi Corpo[illegible]or formerly Mattson-Garvin Company, Homosassa Springs, FL);

- Biotest Reuter Centrifugal Air Sampler (RCS, Biotest Diagnostics Corporation, Denville, NJ);

- Gelman 47 millimeter (mm) Membrane Filter Air Sampler (MF, Gelman Sciences Incorporated, Ann Arbor, MI); and

- Ace Glass All-Glass Impinger-30 Sampler (AGI-30, Ace Glass Incorporated, Vineland, NJ).

1. **Andersen Six-Stage Viable (Microbial) Particle Sizing Sampler (1-STG)**

The Andersen 6-STG sampler is a multi-orifice, cascade impactor with 400 holes per stage, drawing air at a volumetric flow rate of 28.3 liters per minute (lpm). A schematic diagram of this sampler is shown in Figure 1. This sampler can be used to measure the concentration and particle size distribution of bacteria and fungi in the ambient environment. The airborne particles are impacted on the collection medium located a few millimeters below the perforated plate. Viable particles can be collected on a variety of collection media and incubated in situ for counting and identification. Particle velocity increases as the air flows through successively smaller

holes. Large particles, ≥ 7 micrometers (μm), impact on the first stage and smaller particles continue through the sampler 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 deposition in the human lung. Figure 2 shows the relationship of each stage of the sampler with respect to the particular location of deposition in the human respiratory tract.[4]

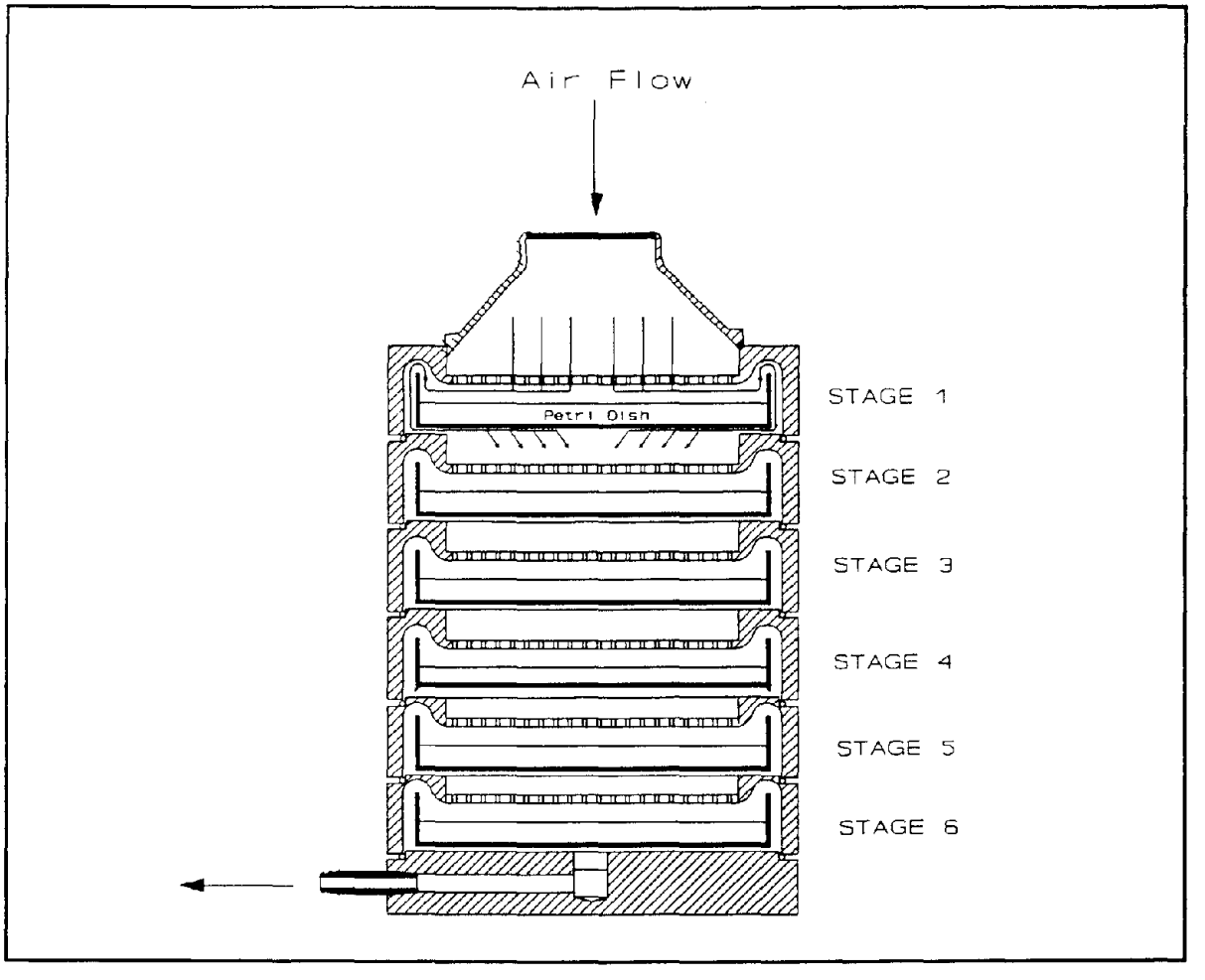

Figure 1. Andersen Six-Stage Viable (Microbial) Particle Sizing Sampler.

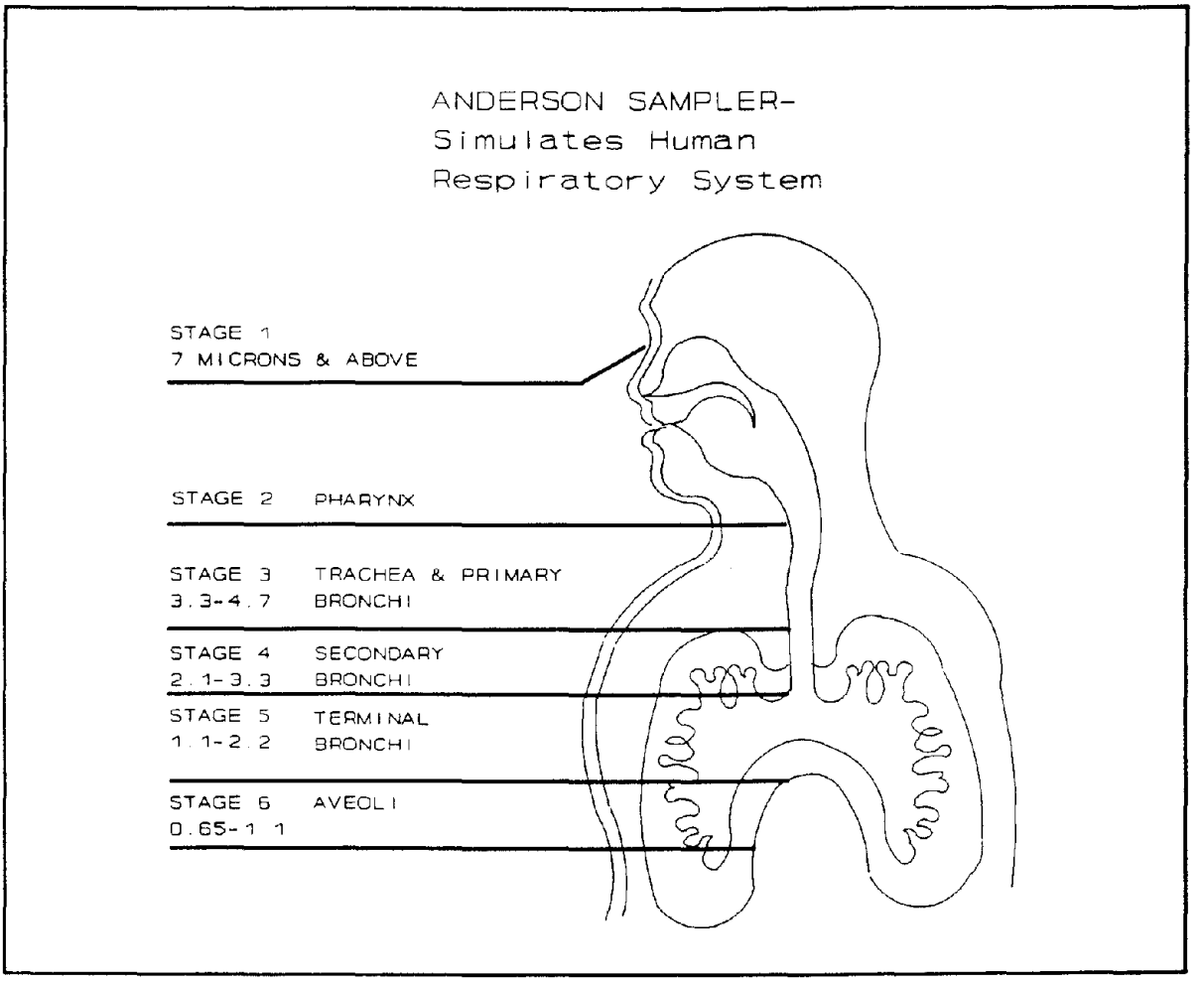

Figure 2. Deposition of Particles in the Human Respiratory Tract.

As a practical matter, these impactors can be assumed to be ideal and the efficiency curves characterized by the Stokes number (Stk_{50}) that gives 50% collection efficiency. This is equivalent to assuming the mass of the particles larger than the cut-diameter (d_{50}) that passes through the impactor equals the mass of particles smaller than the d_{50} that are collected. Hence, the d_{50} is the aerodynamic diameter above which the collection efficiency of the impactor approaches 100%.[5] Figure 3 shows the d_{50} of each stage of the Andersen 6-STG sampler.

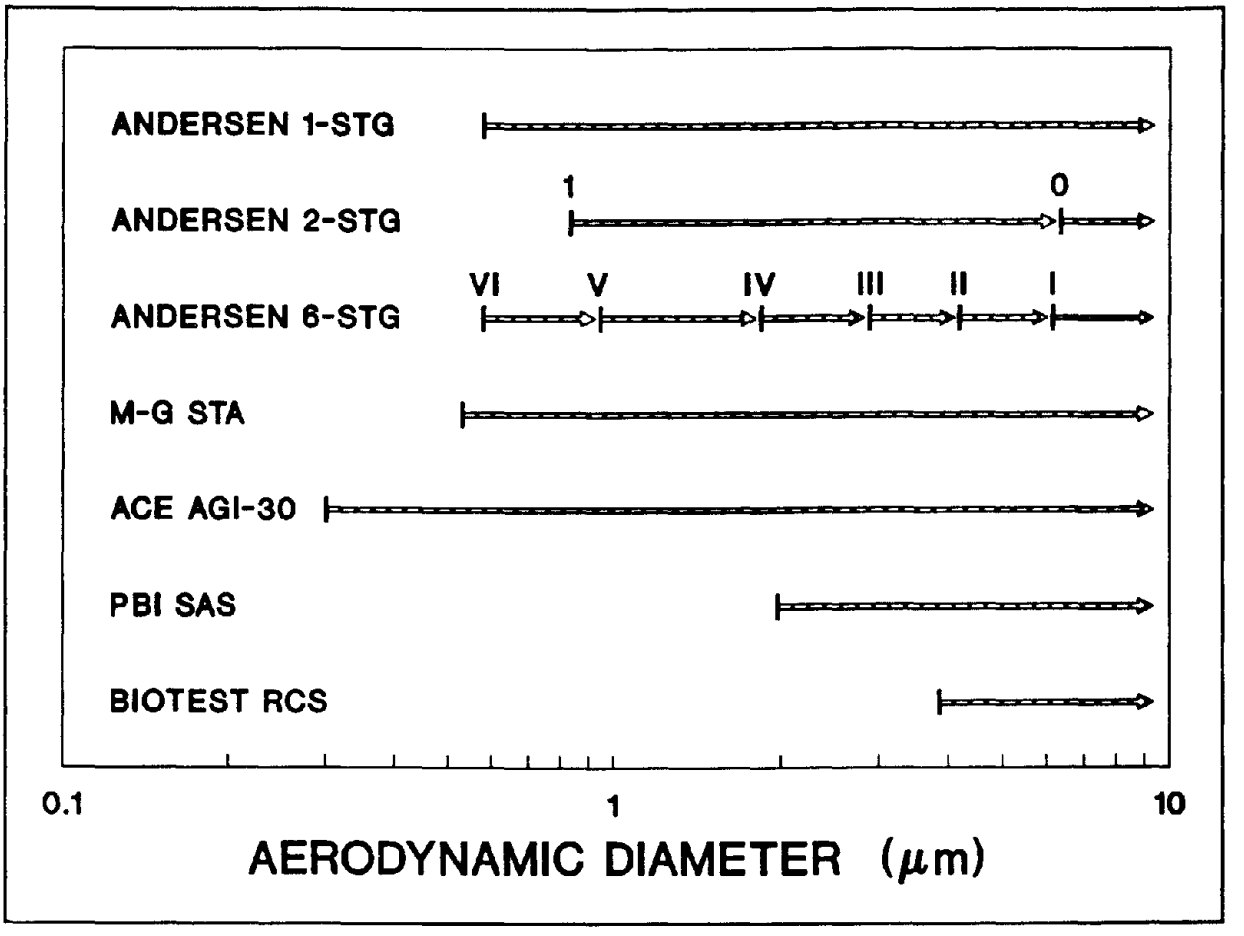

Figure 3. Cut-Off Diameters (d_{50}) for the Seven Bioaerosol Samplers that Operate on the Principle of Impaction.

Usually, impaction methods provide higher particle recovery than other methods. Impaction also results in low sampling stresses and sample manipulation after collection is not required.[3] Sampling plates were prepared by pouring 45 ml of Bacto Tryptic Soy Agar (TSA, DIFCO Laboratories, Detroit, MI) aseptically into each of six 100 mm diameter x 15 mm deep sterile plastic Petri plates so that the gap between the sieve and agar surface met the manufacturer's specification.[6,7] All inside surfaces were maintained sterile until sampling. After sampling for approximately 180 seconds (s) or the equivalent of 84.9 liters (l), the plates were removed from the sampler,

covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 hours (h).

2. Andersen Two-Stage Viable (Microbial) Particle Sizing Sampler (2-STG)

The Andersen 2-STG is also a multi-orifice, cascade impactor with 200 ho ; per stage, drawing air at a flow rate of 28.3 lpm. A schematic diagram s shown in Figure 4. This sampler can be used whenever a size distribution is not required. This unit separates viable particles into two size ranges, with

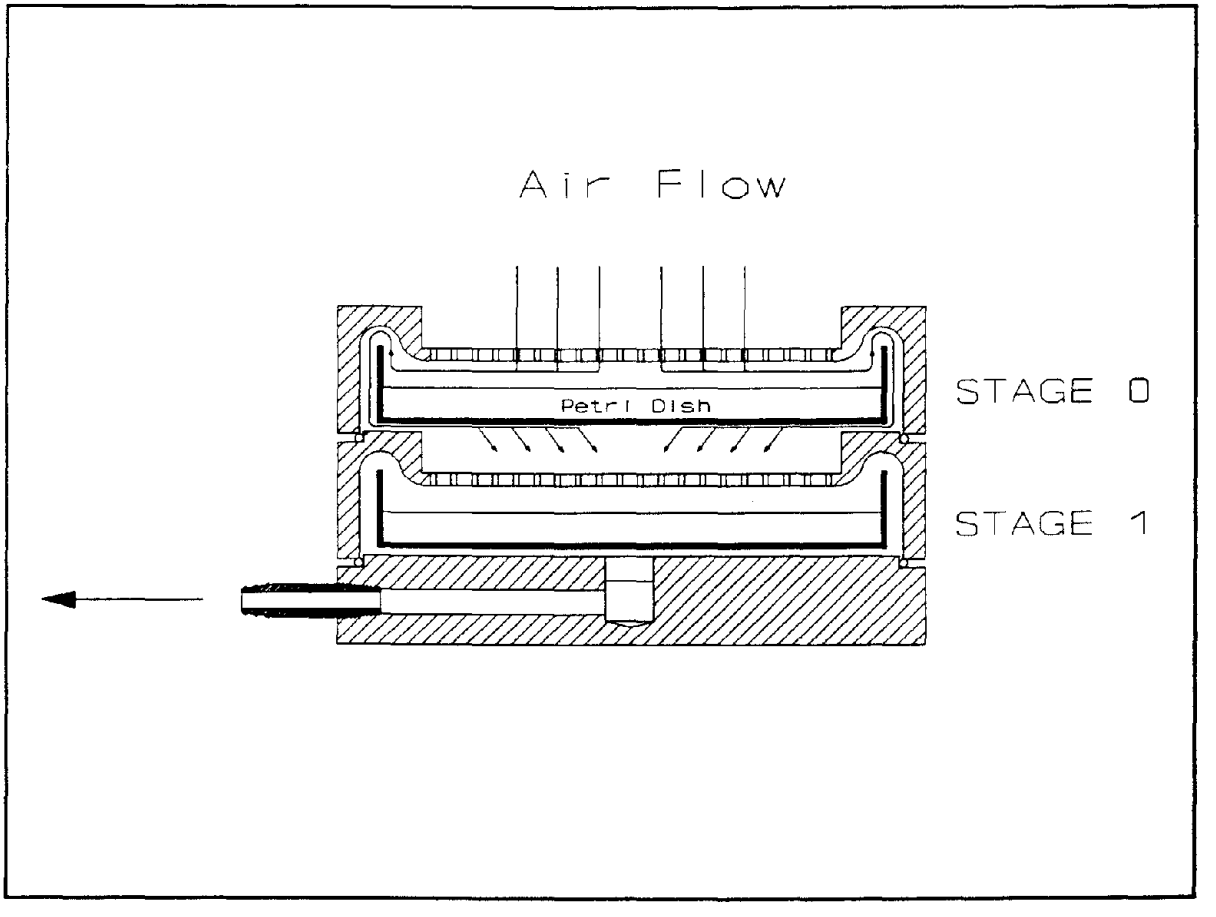

Figure 4. Andersen Two-Stage Viable (Microbial) Particle Sizing Sampler.

the 50% cut-off diameter of Stage I at approximately 8 μm for spherical particles of unit density. According to the manufacturer, 95-100% of the viable particles greater than 0.8 μm in an aerosol can be collected on a variety of collection media. Figure 3 shows the d_{50} of each stage of the sampler. The two Petri plates were each prepared aseptically with 20 ml of TSA so that the gap between the sieve and agar surface met the manufacturer's specification.[6] After sampling for approximately 180 s 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 h.

3. Andersen N6 Single-Stage Viable (Microbial) Particle Sampler (1-STG)

The Andersen 1-STG sampler is the sixth stage of the Andersen 6-STG sampler, drawing air at a flow rate of 28.3 lpm. A schematic diagram is shown in Figure 5. The plate was poured aseptically using 45 ml of TSA so that the gap between the sieve and agar surface met the manufacturer's specifications.[7] Figure 3 shows the d_{50} of the sixth stage of the sampler as 0.65 μm. After sampling for approximately 180 s or the equivalent of 84.9 l, the plate was removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

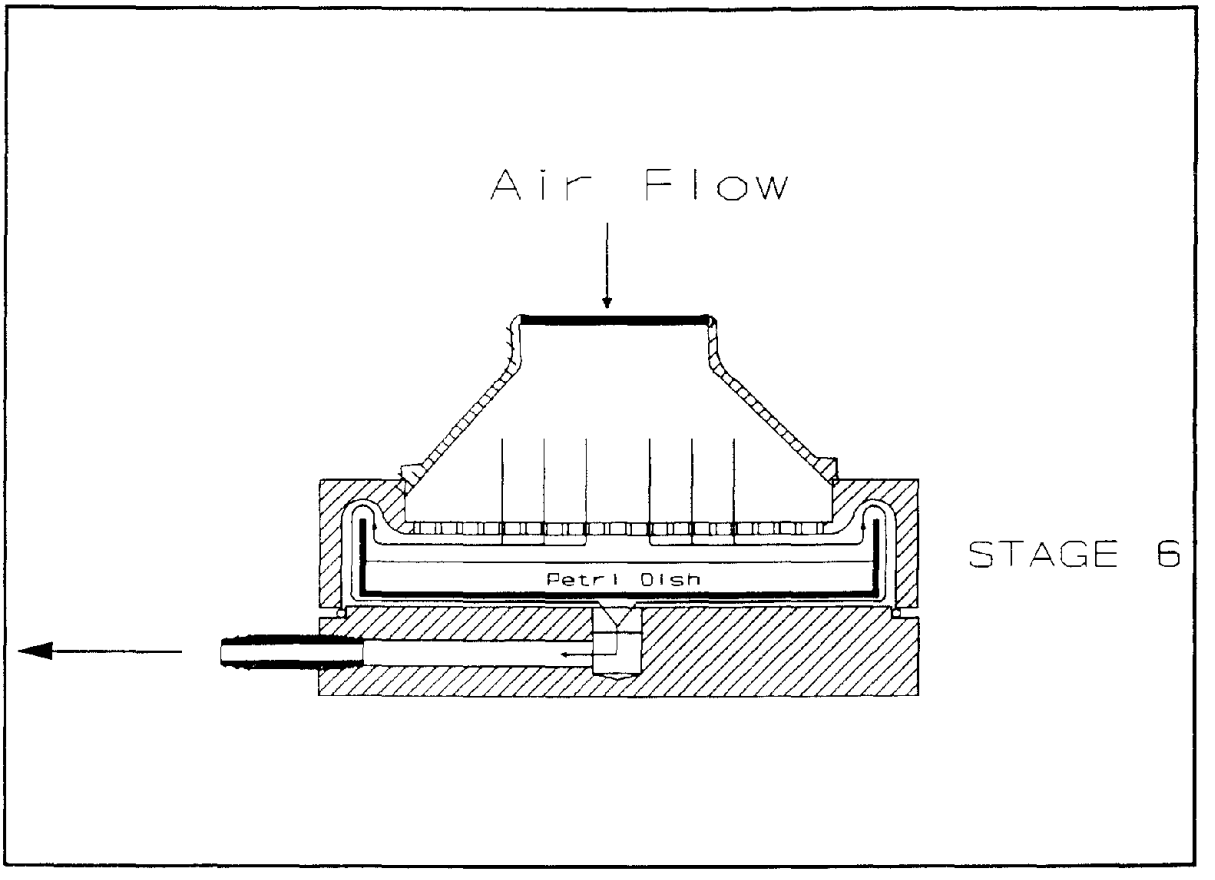

Figure 5. Andersen N6 Single-Stage Viable (Microbial) Sampler.

4. Pool Bionalyse Italiana (PBI) Surface Air System Sampler (SAS)

The PBI SAS sampler operates similarly in principle to the Andersen 1-STG sampler; however, the impactor stage consists of 219 holes, drawing air at a flow rate of 90 lpm. The holes are approximately the same diameter as the second stage of the Andersen 6-STG sampler. A schematic diagram is shown in Figure 6. The SAS sampler has an internal timer that can be set to sample from zero to five minutes, in 20 second increments. A 50 mm Replicate Organism Direct Agar Contact (RODAC) plate was prepared aseptically with TSA so that a convex meniscus formed at the rim of the plate.[8] After sampling

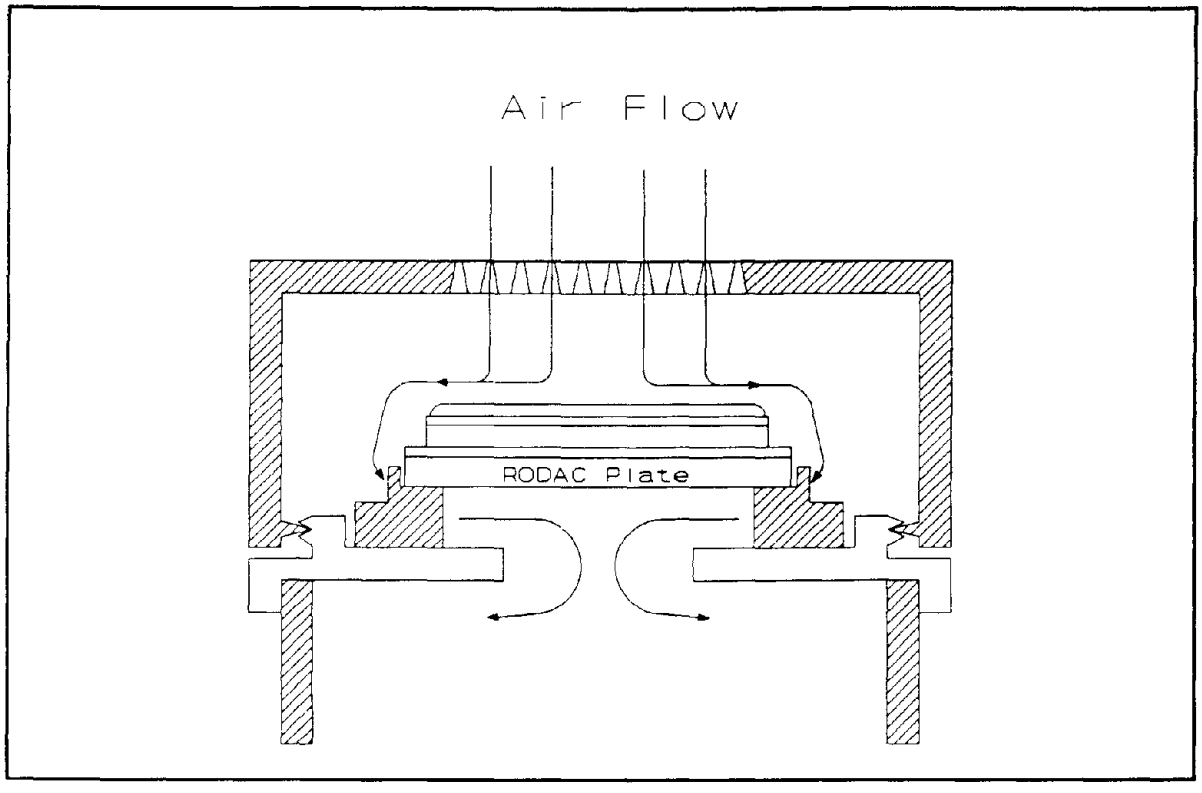

Figure 6. Pool Bionalyse Italiana Surface Air System Sampler.

for approximately 300 s or the equivalent of 450 l, the plate was removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

5. Mattson-Garvin Slit-to-Agar Air Sampler (STA)

The M-G STA sampler uses inertial impaction to collect viable organisms onto a culture medium at a flow rate of 28.3 lpm. Four drive motors of various speeds are provided with this apparatus which result in one rotation of the Petri plate in 60-minutes, 30-minutes, 15-minutes, or 5-minutes. A zero to 60 minute timer is built into the controls. At relatively high concentrations and slow rotation of the Petri plate, the number of colony forming units

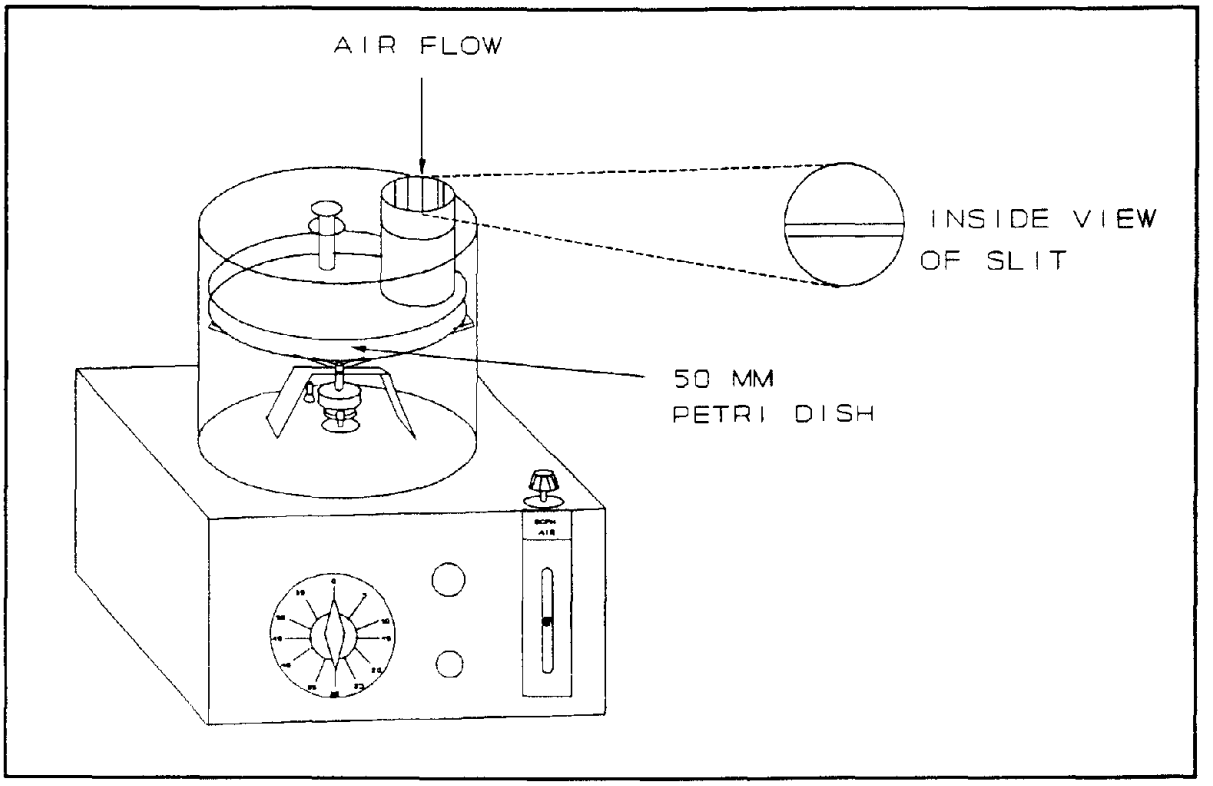

Figure 7. Mattson-Garvin Slit-to-Agar Air Sampler.

impinged upon the agar surface may be too numerous to count. A schematic of this sampler is shown in Figure 7. Figure 3 shows the d_{50} of the STA sampler as approximately 0.5 μm. A 150 mm x 15 mm plastic Petri plate was prepared aseptically with 60 ml of TSA. After the Petri plate was placed on the turntable, the height of the Petri plate was adjusted by fully depressing the slit-to-agar distance gage at the top of the dome assembly and then adjusting the elevation arm on the right side of the instrument so that the media surface touches the gage. This sampler will collect particles which are greater than 0.5 μm in diameter.[9] After sampling using the 5-minute per revolution motor for approximately 180 s or the equivalent of 84.9 l, the plate was removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

6. Biotest Reuter Centrifugal Air Sampler (RCS)

The Biotest RCS also uses inertial impaction to collect viable organisms onto a culture medium. A schematic diagram of this sampler is shown in Figure 8. The manufacturer states that the theoretical flow rate of the sampler is 280 lpm; however, the flow rate was experimentally determined to be approximately 210 lpm by Macher and First.[10,11] Because the air enters and exits the same opening, the actual volumetric flow rate is not easy to determine. Air was impacted onto a commercially available plastic strip containing 34 wells of

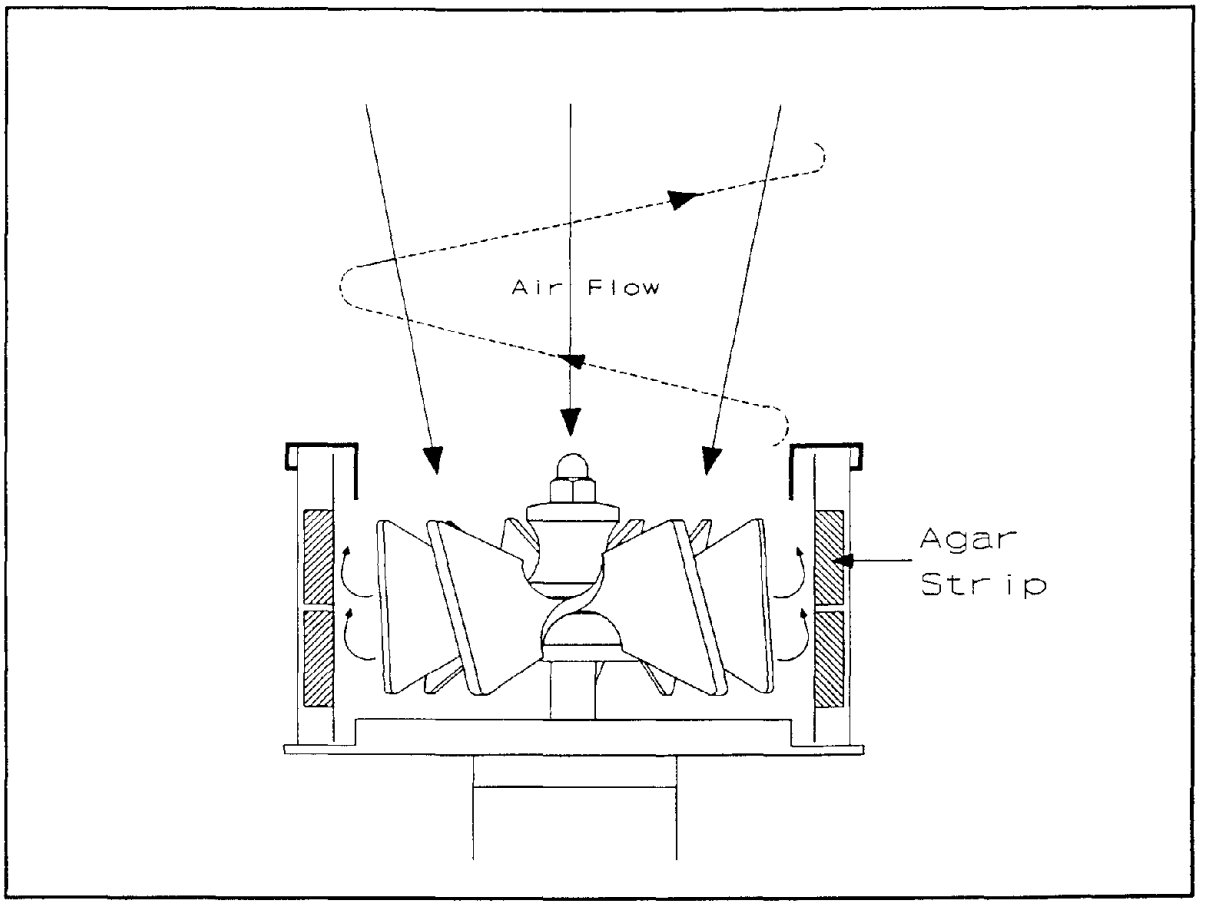

Figure 8. Biotest Reuter Centrifugal Air Sampler.

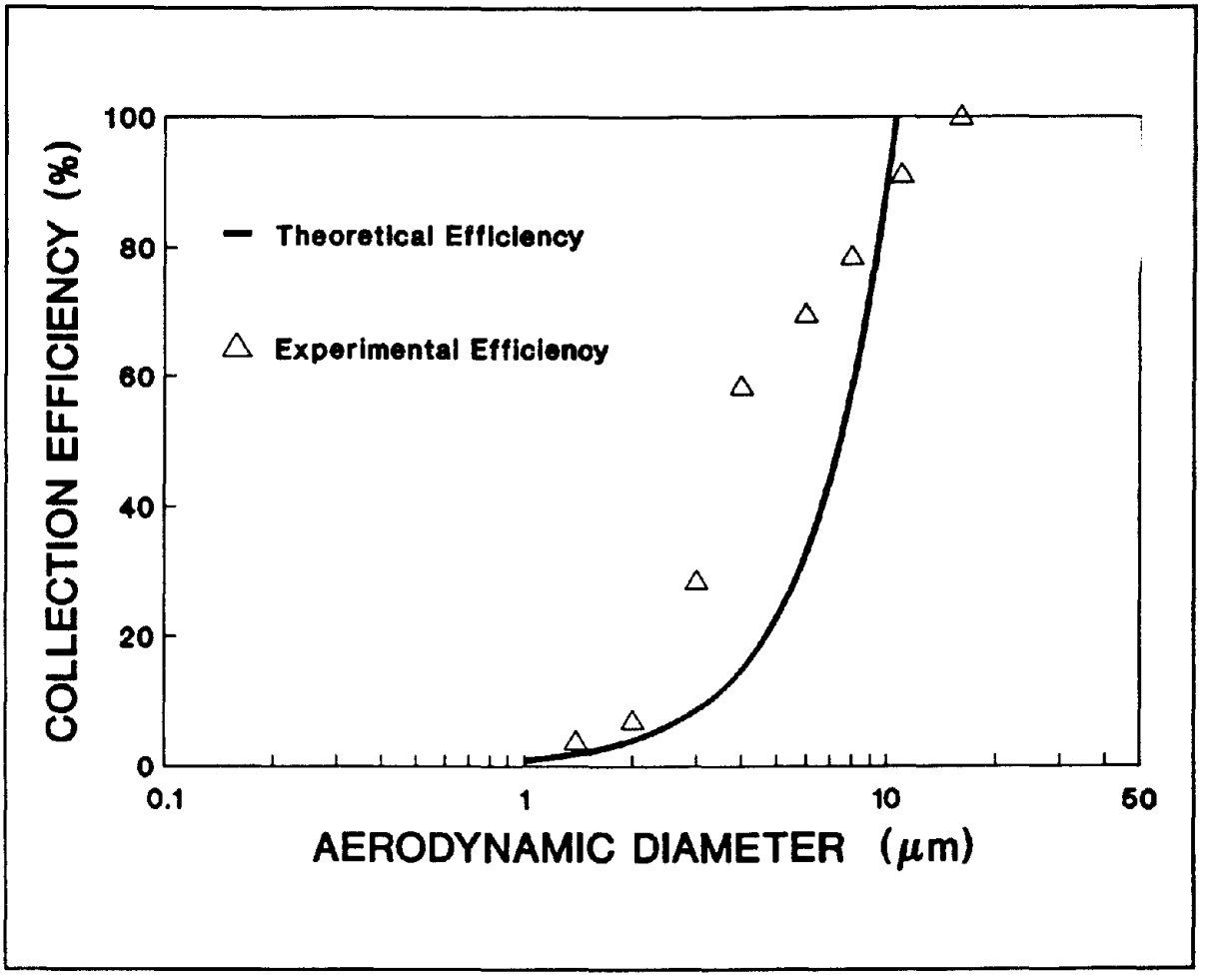

Figure 9. Collection Efficiency of the Biotest Reuter Centrifugal Air Sampler.[10,11]

TSA (Biotest). Each well has an area of approximately one square centimeter. Figure 3 shows the d_{50} of the RCS sampler as approximately 4 μm. After sampling for approximately 480 s or the equivalent of 1680 - 2240 l, the strip was removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

The collection efficiency of this sampler is highly dependent on the particle size of the aerosol being sampled. Figure 9 compares the experimental

collection efficiency, determined by Macher and First, to the theoretical efficiency, determined by Biotest.[10,11] This sampler is a very inefficient sampler for particles in the respirable range.

7. Gelman 47 millimeter Membrane Filter Air Sampler (MF)

The Gelman MF is widely used for aerosol sampling due to its low cost and simplicity of operation. A schematic diagram of this sampler is shown in Figure 10. This air filtration apparatus consists of an aluminum 47 mm open-

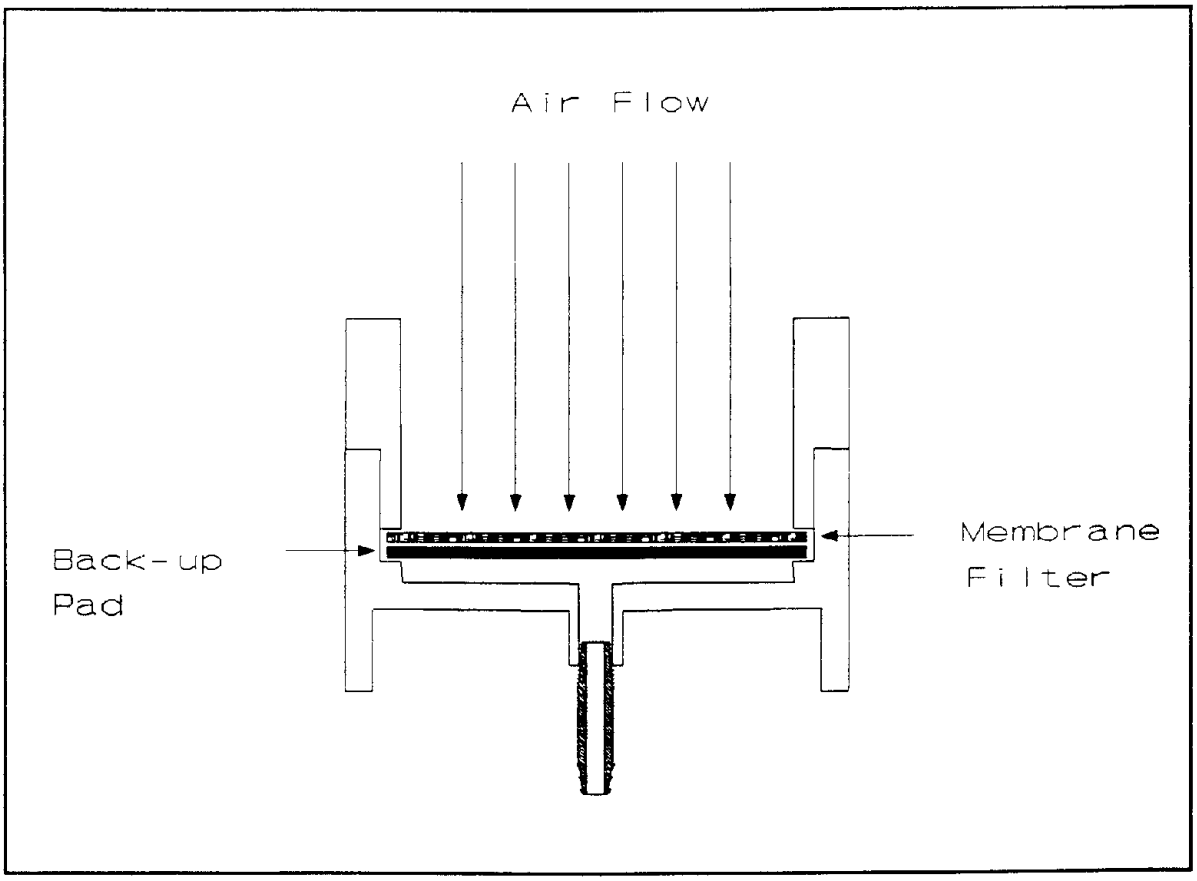

Figure 10. Gelman 47 millimeter Membrane Filter Air Sampler.

faced filter holder with a sterile 47 mm diameter membrane filter made of cellose 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 control er (i.e., critical orifice).[12] After sampling for approximately 480 s at flow rate of 5 lpm, or the equivalent of 40 l, the membrane filter was re ved from the sampler. The membrane filter was placed in a 50 mm Petri plate n a sterile pad saturated with Bacto Tryptic Soy Broth (TSB, DIFCO). The plate with the MF was covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

This membrane filter method is good for enumerating mold and bacterial spores using flow rates of 5-15 lpm. It may not be very effective, even at low flow rates (1-5 lpm), for counting vegetative cells because of the stress of cell dehydration produced during sampling.[13]

8. Ace Glass All Glass Impinger-30 Sampler (AGI-30)

The Ace Glass AGI-30 sampler is a high velocity liquid impinger widely used for air sample collection. A schematic diagram of this sampler is shown in Figure 11. Impinger methods such as this one use a liquid (simple salt solutions, with additives such as proteins, antifoam, or antifreeze) as the collection medium. The jet is positioned 30 mm above the impinger base and consists of a short piece of capillary tube designed to reduce cell injury when the air is dispersed through the liquid and entrapped. The AGI-30 sampler operates by drawing aerosols at a flow rate of 12.3-12.6 lpm through an inlet tube curved to simulate the nasal passage.[14] This makes it

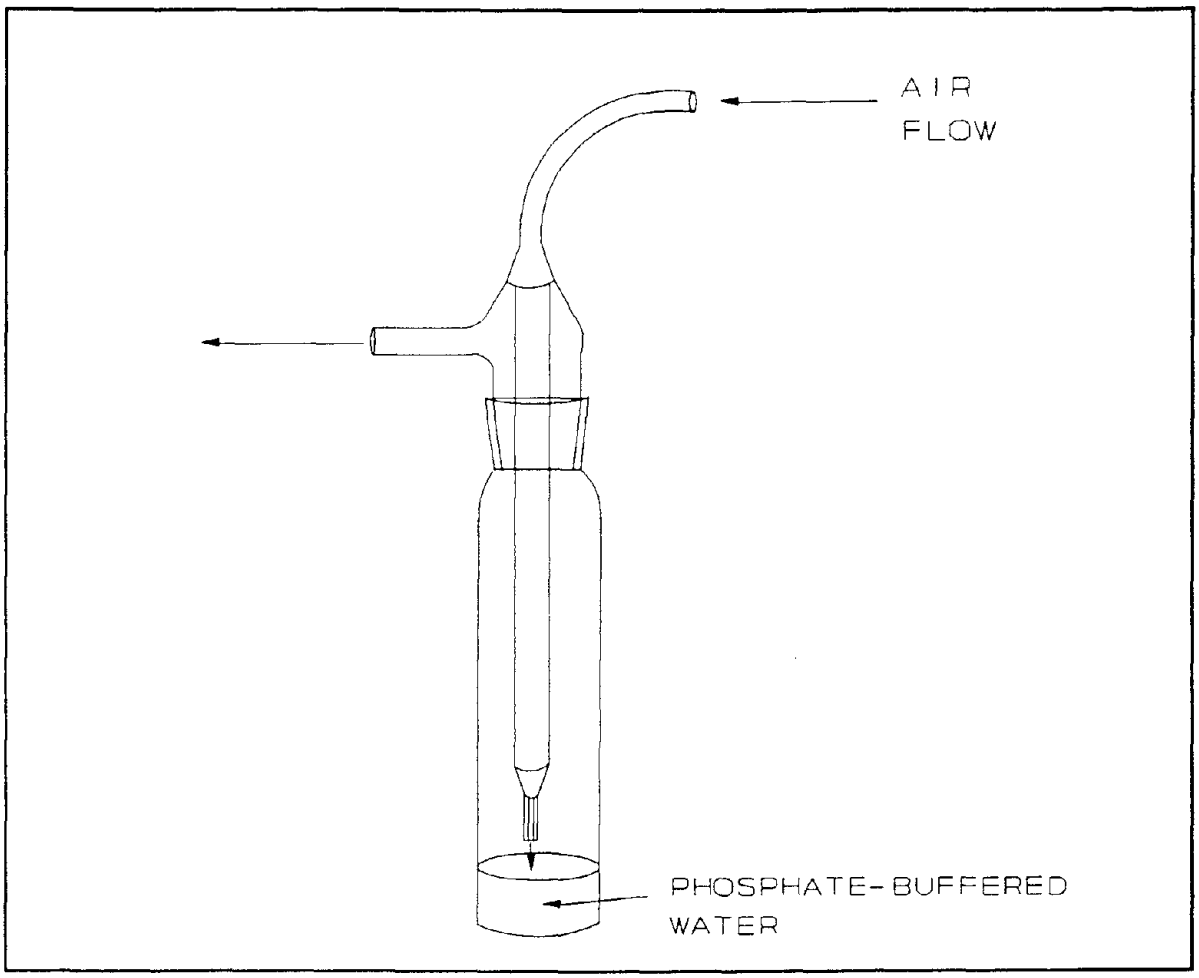

Figure 11. Ace Glass All Glass Impinger-30 Sampler.

especially useful for studying the respiratory infection potential of airborne microorganisms. When it is used to recover total airborne organisms from the environment, the curved inlet tube should be washed with a known amount of collecting fluid after sampling because larger particles (i.e., over 15 μm) are collected on the tube wall by inertial force. After sampling for approximately 180 s or the equivalent of 37.5 l, quantifying of airborne microbes was accomplished by using a membrane filtration plating technique because the expected microbial load was low.[15] The membrane filter was placed in a 50 mm Petri plate on a pad saturated with TSB. The plate with the

MF was covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

The AGI-30 sampler is inexpensive and simple to operate, but viabili' .oss may occur due to the amount of shear force involved in collection. e airstream approaches sonic velocity when particles impinge on the c .lection medium, resulting in almost complete collection of suspended particles. This condition, however, tends to cause the destruction of some vegetative cells or may result in overestimation due to the dispersion of dust particles and the breaking up of clumps of bacteria[16,17,18] After sampling high concentrations of bioaerosols, quantifying of airborne microbes may be quantified by diluting and plating the collection fluid on nutrient medium. The plate is then incubated at the appropriate temperature and the colonies are counted.

B. 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).[19] The volumetric flow rate through each of these sampling instruments was verified before each day of sampling 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. Sampling rate was found to be within 10% of the manufacturer's specification (90 lpm). The RCS is not easy to calibrate or even verify the flow rate.[11] The manufacturer's recommendations were followed by use of the Calibration-Set (Biotest) to check and if necessary correct the impeller blade angle. In addition, the internal timer was verified to be within manufacturer's specification (8-minutes ± 2%) using a stopwatch.

C. 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.[20] The chamber was a stainless steel 0.61 m square duct, approximately 3.7 m long. Two baffles were located down stream of the aerosol inlet to promote uniform mixing of the aerosol with the flow stream. 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 through the roof of the laboratory by an existing stack. Four side hatches provided for easy access to the inside of the chamber to place and remove samplers, and for the application of chemical disinfectants. A schematic diagram of this Bioaerosol Chamber is shown in Figure 12. Further details are outlined in reference 21.[21]

The flow rate of air though the chamber was adjustable, but for the purpose of this study, the flow rate was maintained at 11.3 m^3/min. This flow rate

resulted in an air velocity of approximately 0.54 m/sec. Vertical and horizontal velocity traverses were performed using a hot-wire anemometer. The velocity throughout the cross-sectional area of the sampler location was uniform and was determined to be 0.54 m/sec ± 0.014 m/sec. To verify that e aerosol was uniformly distributed across the same cross-sectional area, a mono-disperse aerosol of oleic acid was generated using a Berglund-Liu Vibrating Orifice Monodisperse Aerosol Generator.[22,23] An aerosol with an aerodynamic diameter of approximately 5 μm entered the chamber through the

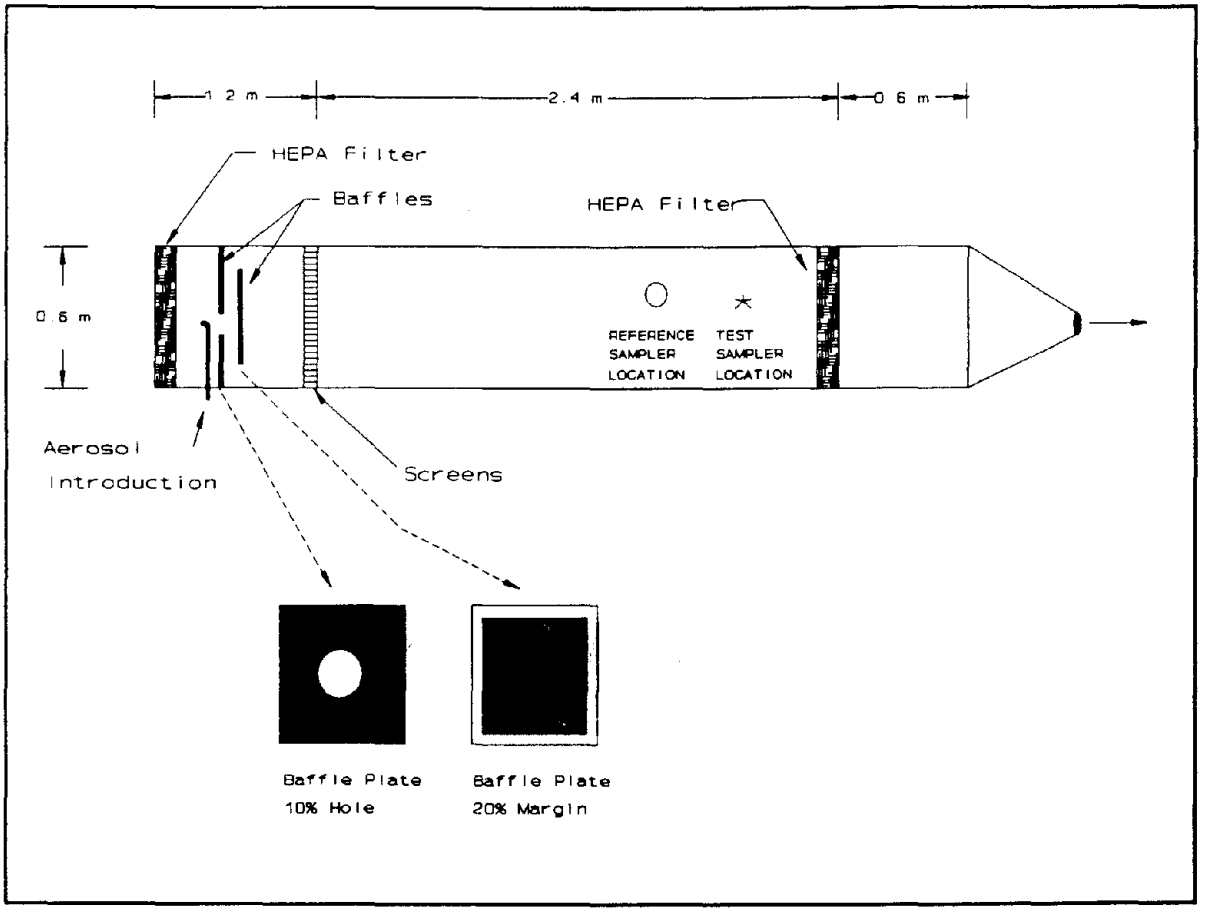

Figure 12. Diagram of the NIOSH Bioaerosol Chamber.

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 using a TSI® Aerodynamic Particle Sizer (APS, TSI Incorporated, St. Paul, MN) and found to be 5.37 $\mu m \pm 1.13$ μm. The mass concentration for these traverses was measured to be 0.126 $mg/m^3 \pm 0.035$ mg/m^3.

D. Preparation of Pure Culture Suspensions

Cultures used included E. coli (ATCC 11229) and B. subtilis (ATCC 6633, DIFCO). E. coli is a Gram negative, non-spore forming bacteria which is approximately 0.5 μm wide and 2.0 μm long. B. subtilis is a Gram positive, endospore-forming bacteria of similar size and shape. Active E. coli and B. subtilis cultures were inoculated into approximately 100 ml of TSB and incubated for 18-24 hours 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, NJ), capped, and centrifuged at 1500 g for 10 minutes in an IEC CENTRA®-4B General-Purpose Centrifuge (International Equipment Company, Needham, MA). 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. The cell concentration was determined by turbidimetric measurement of this suspension. The transmittance of monochromatic light at a wavelength of 450 nanometers through the cell

suspension was measured using a Spectronic® 20 spectrophotometer (Milton Roy Company, formerly Bausch & Lomb, Rochester, NY). The concentration of cells was estimated from a previously developed calibration curve for the spectrophotometer, and the appropriate dilution was made to attai a total volume of 100 ml with a concentration of approximately 10^6 bacte: al cells per milliliter of solution for use in the aerosol generator.[24] A di_ution series was performed for standard plate count enumeration.[25] The nebulizer then generated an aerosol of mostly single bacteria cells with a chamber concentration of approximately 2000 CFU/m^3.

E. Aerosol Generation

A Collison nebulizer was used to aerosolize the suspensions of E. coli or B. subtilis cells. A schematic of the BGI 6-jet modified MRE-type Collison Nebulizer (BGI Collison Nebulizer, BGI Incorporated, Waltham, MA) is shown in Figure 13. The BGI 6-jet modified MRE-type Collison Nebulizer (BGI Collison Nebulizer, BGI Incorporated, Waltham, MA) was used in this study. Within the nebulizer, compressed air expanded from a pressure of 140 kilopascals (kPa) at the stem into the six side jets. The reduction of static pressure forced water up the tube at the bottom of the stem, similar to an eductor. This fluid 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 wall of the glass vessel; however, the minute amount of liquid which escaped impact comprised only the finest tail of the drop-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

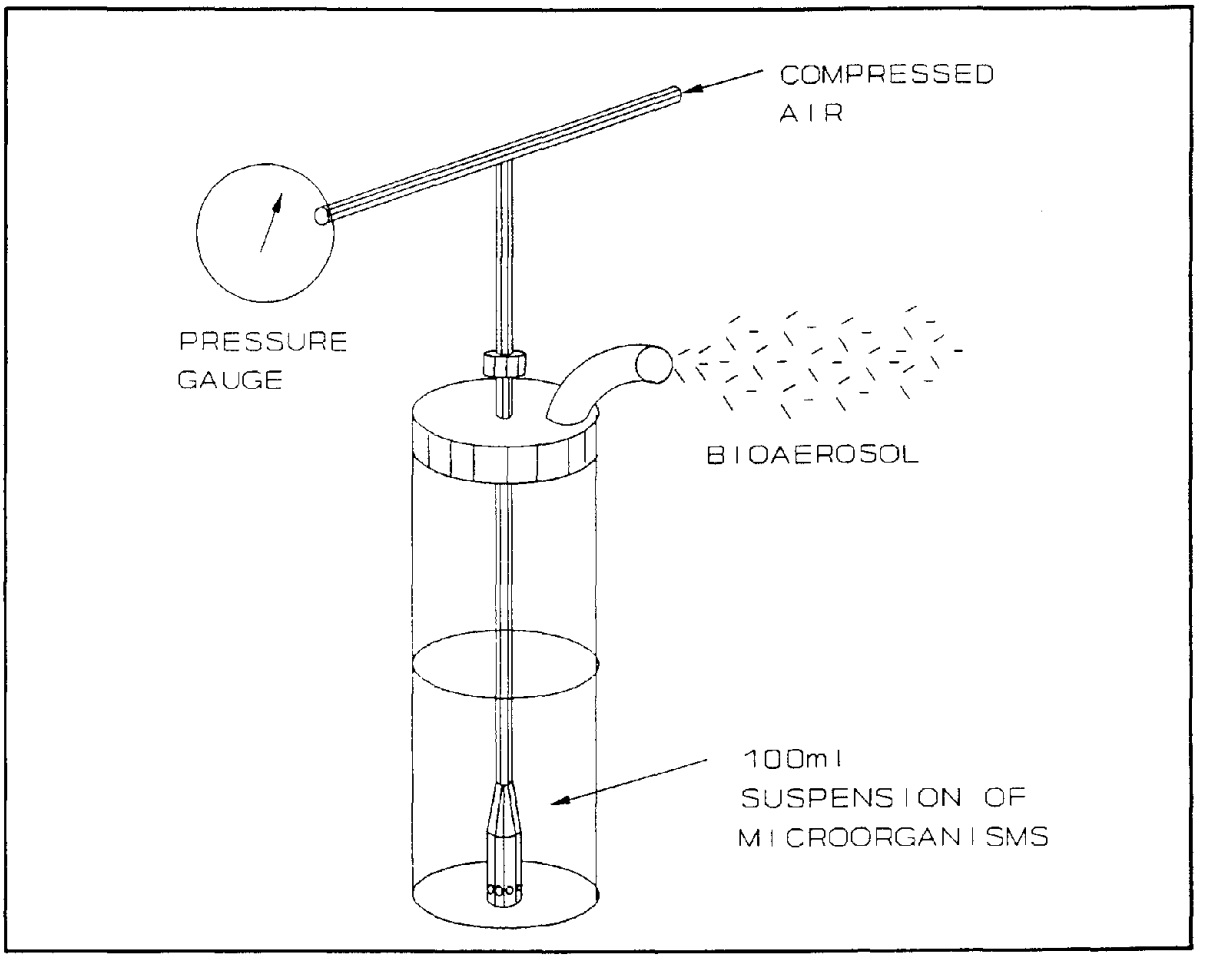

Figure 13. BGI 6-Jet Modified MRE-Type Collison Nebulizer.

on admixture with unsaturated air. For example, a 10 μm water droplet in air at 20°C and 80% RH has a wet lifetime of 0.6 seconds; where as a 2.9 μm water droplet has a wet lifetime of 0.03 seconds. The wet lifetime is proportional to the square of the diameter at a given temperature and relative humidity.[26] 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.[27] Theoretically, mostly single bacterial cells make their way to the sampler location in the aerosol chamber, a transit time of approximately six seconds or approximately 10 life times for

a 10 μm water droplet. To validate the assumption that only particles containing either one bacteria cell and water, or just water, particles from the Collison nebulizer were collected on a glass slide and viewed using a phase contrast microscope (LABOPHOT-2, Nikon Corporation, Tokyo, Japan). No clumping of bacteria was observed.

The distributions of CFUs collected on the Andersen 6-STG sampler for both E. coli and B. subtilis are shown in Figure 14. The distributions indicate that a majority of the particles collected had an d_{ae} between 1.1 and 2.1 μm.

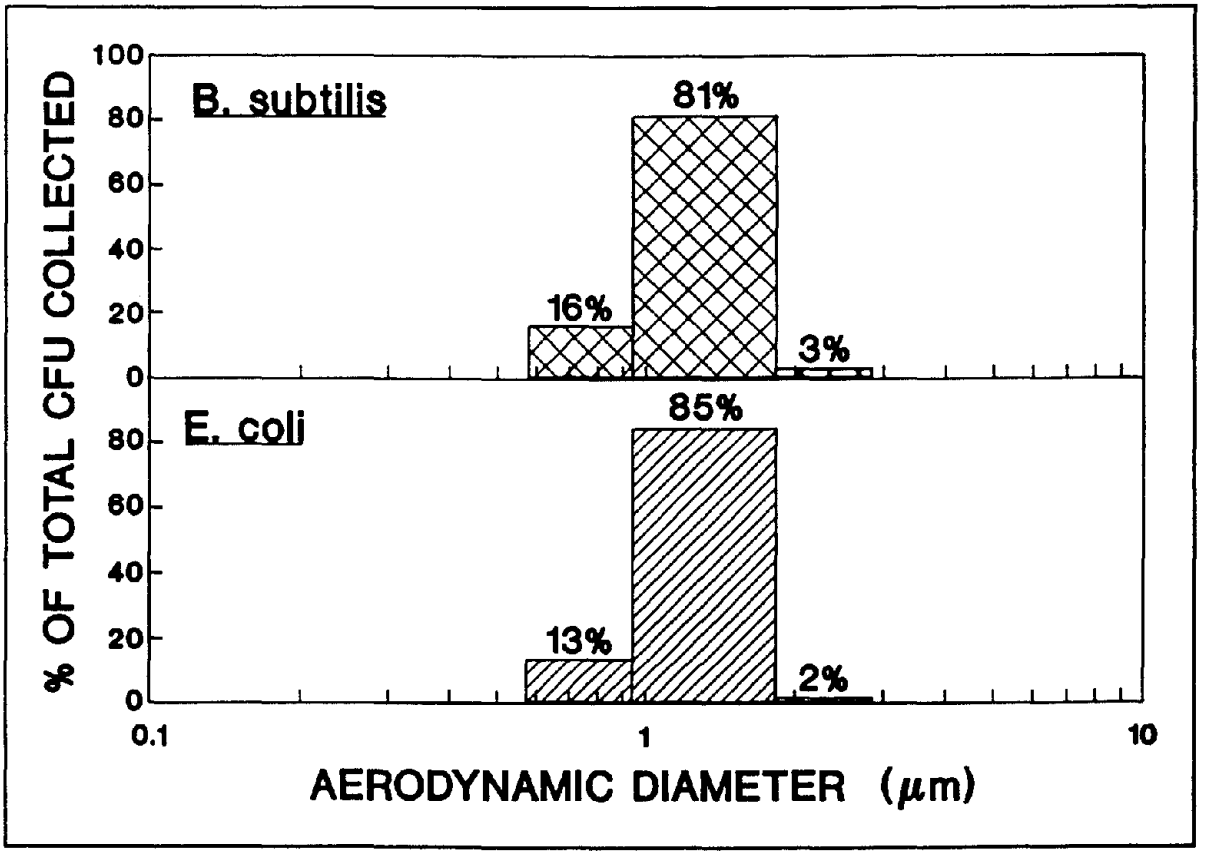

Figure 14. Size Distribution of E. coli and B. subtilis Aerosols, Collected on an Andersen 6-STG Sampler.

The viability of E. coli and B. subtilis in the nebulizer was tested independently. A 100 ml suspension of approximately 10^6 cells/ml of phosphate-buffered dilution water was placed both in a sterile nebulizer jar and in a sterile Collison nebulizer. The nebulizer was cycled (five minutes on and five minutes off for the first 90 minutes) and aliquots of the nebulized solution were diluted and plated. In parallel, aliquots of the suspension (static cells) were also diluted and plated. As shown in

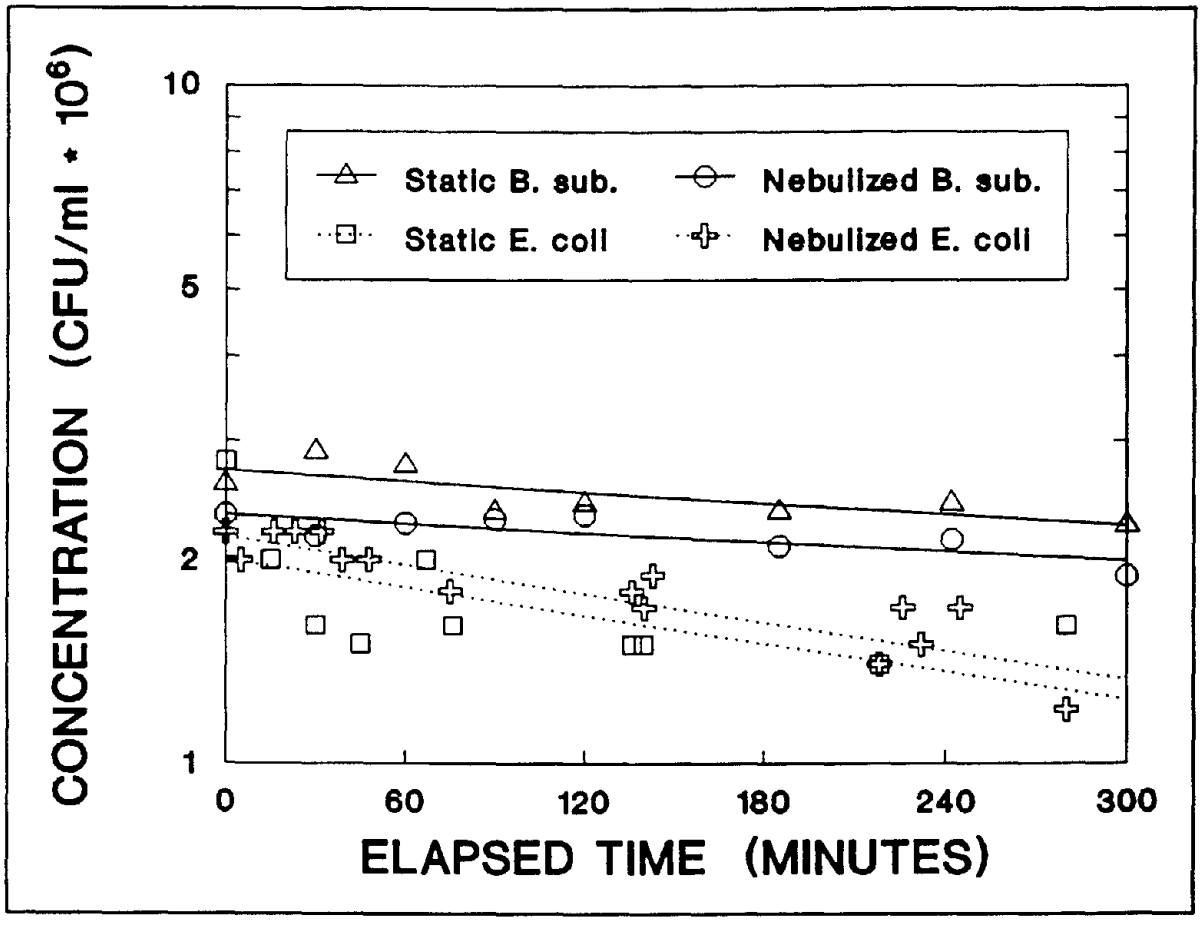

Figure 15. Comparison of the Viability of E. coli and the Viability of B. subtilis in Phosphate-Buffered Dilution Water when Nebulized and when in a Static Environment.

Figure 15, 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 five hours indicating that E. coli and B. subtilis remain viable in phosphate-buffered dilution water for extended periods of time.

F. Enumeration of Bacteria

When the concentration of viable particles in an aerosol is high, one sieve hole may allow more than one viable particle to pass through resulting in the formation of a single colony from two or more viable particles. This inaccuracy can be corrected by reducing sampling time or by using either the microscopic method or a "positive hole" method for enumeration. The microscopic method involves counting particles using a dissecting-type microscope before the colonies merge. The "positive hole" method, designed for the Andersen 6-STG, 2-STG, and 1-STG samplers, is essentially a count of the jets which delivered viable particles to the Petri plates. This count is converted to a viable particle count by the use of the "positive hole" coincidence conversion tables.

For this study, all plates were enumerated using a New Brunswick Model C-110W Colony Counter (New Brunswick Scientific Co., Inc., Edison, NJ) after incubation for 24 h at a temperature of 35°C in a Forma Scientific Water Jacketed Incubator Model 3158 (Forma Scientific, Marietta, OH). All CFUs on each plate were counted, which included a number of "multiple hits". Because it was very easy to identify more than one CFU at an impaction point, we did

not apply a positive hole statistical adjustment. Detailed discussions of the positive hole statistical adjustment are found in the literature.[4,28,29]

III. OBJECTIVES AND HYPOTHESIS

The primary objective of this phase of the IA was to determine the precision of alternative bioaerosol sampling methods and the relative collection efficiencies of these alternative bioaerosol sampling methods when challenged with an aerosol containing viable microorganisms. The Andersen Six-stage Viable 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 ACGIH. The resultant data were used to develop a series of models to compare each instrument to these two samplers of choice (i.e., 6-STG and AGI-30). Assessments were made concerning the feasibility and applicability of using these alternative samplers for use in the biotechnology industry to determine process microorganism releases in fermentation type operations.

IV. EXPERIMENTAL DESIGN

The eight microbial samplers were compared to an AGI-30 sampler located approximately 0.6 meters upstream of the 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 and/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 the AGI-30 has a calculated d_{50} of 0.30 μm. Four samplers were tested during each sampling session for a total of 10 sessions, with only one sampling session per sampling day. The order in which the samplers were tested was randomized. Samples were taken with four replications.

V. EXPERIMENTAL CONFIGURATION/CONDITIONS

To accomplish the primary objective, dependent and independent variables affecting each sampler's ability to collect microorganisms in air are identified below:

Dependent variables: Microorganism concentration as determined by each of the following test samplers:

- Andersen 6-STG
- Andersen 2-STG
- Andersen 1-STG
- PBI SAS
- M-G STA
- Biotest RCS
- Gelman MF
- Ace Glass AGI-30

Microorganism concentration as determined by the following reference sampler located upstream of test samplers:

- Ace Glass AGI-30

Independent variables: Air velocity - controlled to 0.54 meters per second;

Air temperature - controlled (21-24 °C), continuously monitored;

Air relative humidity - controlled (45-55 %RH), continuously monitored;

Concentration of microorganism in chamber - concentration of microorganism in the chamber is directly related to the concentration of microorganism in the nebulizer. The concentration nebulized was estimated prior to nebulization to assure an air concentration of approximately 2000 CFU/m^3.

Microbial viability - microorganisms were suspended in sterile buffer solution to maintain viability and inhibit growth.

Sampler sterility - vegetative and non-vegetative microorganisms on critical contact surfaces were eliminated by autoclaving or use of chemical disinfectants.

Operator sampling technique - Aseptic techni commonly used in microbiology were used for sample collection, handling, storage, and analyses.

VI. RESULTS

A. Overall Evaluation of Eight Bioaerosol Samplers

All statistical analyses were performed using SAS Version 6.03.[30] 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), all 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 "SAMPRATIO." Analysis of Variance (ANOVA) tables were generated using the General Linear Models Procedure (PROC GLM). The dependent variable, SAMPRATIO, was regressed with three independent variables: sampler type; day of evaluation; and sequence within each day. The means and the 95% confidence intervals for the concentration ratios are shown in Figure 16 and Figure 17.

The results of the multiple comparison tests of SAMPRATIO for each sampler are shown in Table I and Table II. 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

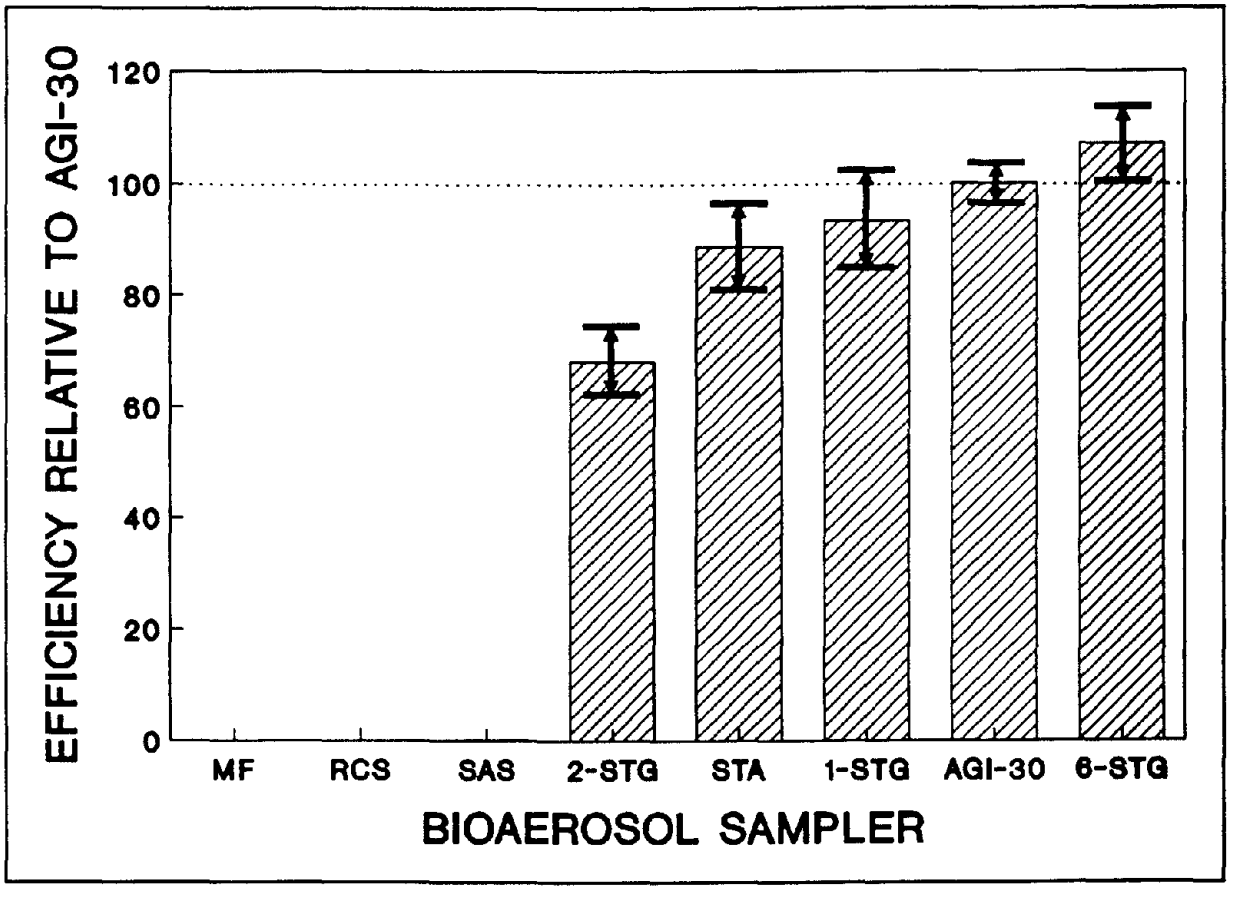

Figure 16. Recovery of Aerosols of E. coli Using Eight Bioaerosol Samplers. Arithmetic Mean of the Concentration Ratio of the Test Sampler to the Reference Sampler, in Percent, and the 95% Confidence Limits About the Mean.

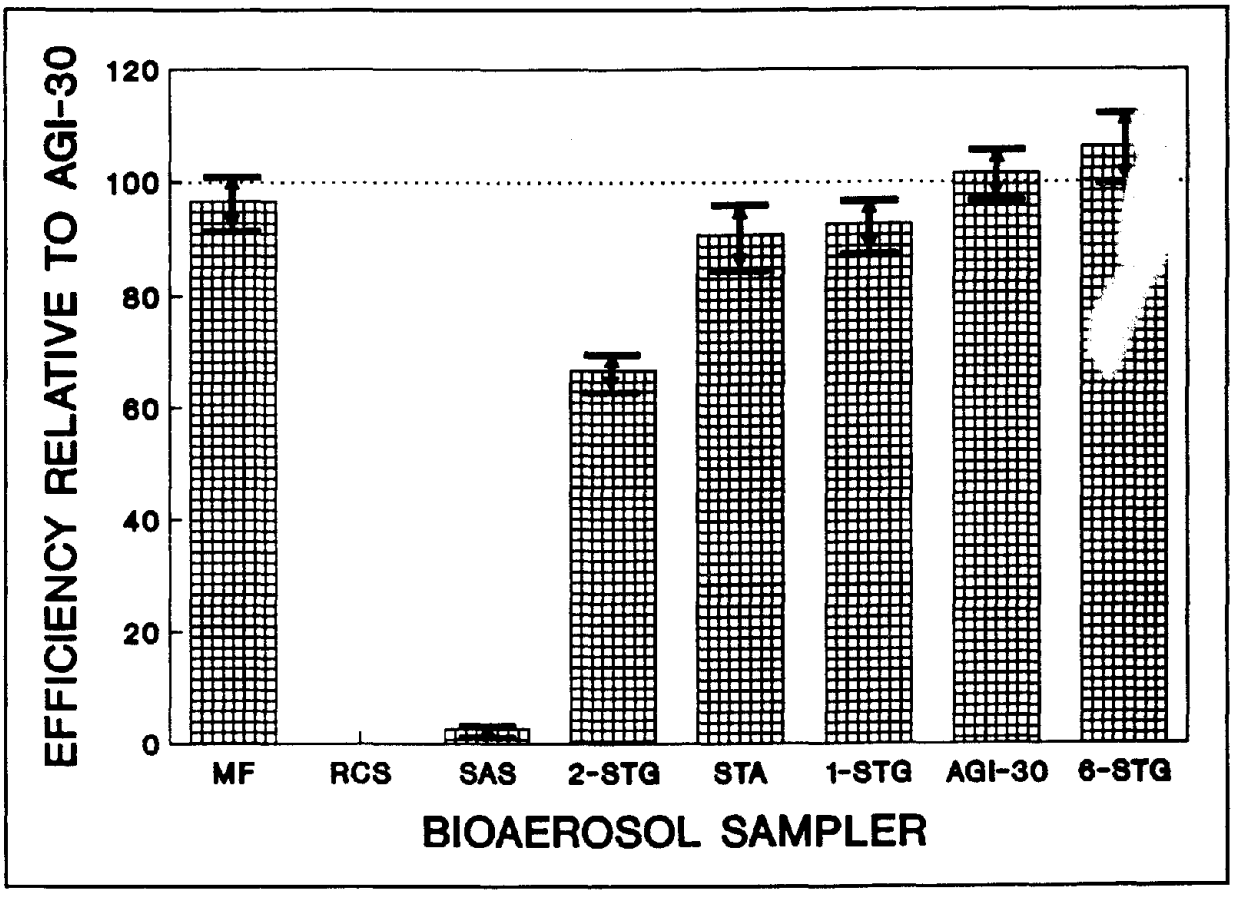

Figure 17. Recovery of Aerosols of B. subtilis Using Eight Bioaerosol Samplers. Arithmetic Mean of the Concentration Ratio of the Test Sampler to the Reference Sampler, in Percent, and the 95% Confidence Limits About the Mean.

different: 6-STG and AGI-30; AGI-30, MF, and 1-STG; MF, 1-STG, and STA; and SAS and RCS.

B. Recovery of Vegetative Cells vs. Bacterial Endospores

Data shown in Figure 16 and Figure 17 compare the recovery of free bacteria aerosols of vegetative cells (E. coli) and bacterial endospore-formers (B. subtilis), respectively. A major difference between the vegetative cells and the bacterial endospores was observed in the performance of the MF sampler.

The low relative efficiency of the MF with E. coli was likely due to the desiccation of the E. coli on the membrane filter. B. subtilis, an endospore-former, is more resistant to desiccation, thus the relative collection efficiency using the MF is similar to that of the reference AGI-30.

Table I. Bonferroni (Dunn) *t* Tests of the Variable SAMPRATIO for Eight Bioaerosol Samplers Challenged with an Aerosol of Escherichia coli.

Bonferroni Grouping		Mean	N	Sampler
	A	107.1	20	6-STG
	A			
B	A	100.1	20	AGI-30
B				
B	C	93.4	20	1-STG
	C			
	C	88.7	20	STA
	D	68.1	20	2-STG
	E	0.089	20	SAS
	E			
	E	0.028	20	RCS
	E			
	E	< 0.001	20	MF

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

Table II. Bonferroni (Dunn) *t* Tests of the Variable SAMPRATIO for Eight Bioaerosol Samplers Challenged with an Aerosol of Bacillus subtilis.

Bonferroni Grouping		Mean	N	Sample
	A	105.9	20	6-STG
	A			
B	A	101.4	20	AGI-30
B				
B	C	96.6	20	MF
B	C			
B	C	92.6	20	1-STG
	C			
	C	90.7	20	STA
	D	66.7	20	2-STG
	E	2.59	20	SAS
	E			
	E	< 0.001	20	RCS

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

VII. 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 confirms other reports in the literature.[31,32,33,34,35] Usually, impaction methods give higher particle recovery than other methods. Impaction onto the nutrient medium located a few millimeters below the perforated plate results in low sampling stresses, provided sampling times are kept as short as possible (< 30 minutes for most organisms) to prevent desiccation of the cells collected on the medium. Post-collection sample manipulation is not required with this method.

Viability loss may occur in the AGI-30 due to 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, however, tends to cause the destruction of some vegetative cells or may result in overestimation due to the dispersion of dust particles and the breaking up of clumps of bacteria.[34,35,36]

The relative sampling efficiency of the Mattson-Garvin STA was marginally lower than the others. Because of the low d_{50} (0.5μm), the STA should have a relative sampling efficiency similar to the 6-STG, 1-STG, and AGI-30.

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. By superimposing Figure 3 on Figure 14, it

is clear that the lower tail of the aerosol distribution is below the d_{50} of the 2-STG, which explains the lower relative efficiency.

The PBI SAS and Biotest RCS samplers collected very few microorganis . The collection efficiency of these samplers is dependent on the partic size of the aerosol being sampled. For the RCS, both the experimental co. ection efficiency for small particles (< 2μm) determined by Macher and First and the theoretical efficiency for small particles calculated using the empirical formula developed by Biotest indicate the poor collection characteristics of this instrument. With a d_{50} of 3.8 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.[10,11] The advantage of portability of these two instruments does not outweigh the inefficiency of these instruments to sample free bacteria. These samplers have shown better recovery of larger particles.[8,11]

The vegetative cells of E. coli were essentially killed by desiccation using the Gelman MF sampler while 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.[37,38]

The results of this study indicate that the Andersen 6-STG, Andersen 1-STG, and Ace Glass AGI-30 samplers are 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.[3,39,40] Recently, the Andersen 1-STG

has been suggested by the American Conference of Governmental Industrial Hygienists Committee on Bioaerosols as a sampler of choice.[40] 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.

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APPENDICES

A. Data from *E. coli* and *B. subtilis* experiments.

B. Prepublication copy of: Jensen, P.A.; Todd, W.F.; Davis, G.N.; Scarpino, P.V.: Evaluation of Eight Bioaerosol Samplers Challenged with Aerosols of Free Bacteria. Submitted for Publication. Am. Ind. Hyg. Assoc. J., 1991.

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C. Prepublication copy of: Martinez, K.F.; Jensen, P.A.; Todd, W.F.; Heitbrink, W.A.: Design and Evaluation of an Aerosol Chamber for Testing of Aerosol Samplers. Submitted for Publication. Am. Ind. Hyg. Assoc. J., 1991.

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Evaluation of Eight Bioaerosol Samplers Challenged with Aerosols of Free Bacteria

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Abstract

The need to quantify airborne microorganisms in the commercial microbiology industry (biotechnology) and during evaluations of indoor air quality (IAQ), infectious disease outbreaks, and agriculture health investigations have shown there is a major technological void in bioaerosol sampling techniques to measure and identify viable and non-viable aerosols. As commercialization of microbiology increases and diversifies, it is increasingly necessary to accurately assess occupational exposure to bioaerosols. Exposure estimates, using area or environmental samplers, can only be assured by the generation of data that are both precise and accurate. The Andersen Six-stage Viable 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 ACGIH. 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, $d_{ae} \leq 2$ μm). The particle concentration in the aerosol chamber as indicated by the AGI-30 sampler was 2000 ± 270 Colony Forming Units per cubic meter (CFU/m^3). The collection efficiency of the evaluated AGI-30 relative to the reference AGI-30 was 100% ± 4%. The 6-STG over-sampled 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 under-sampled the reference AGI-30 by 8%, 11%, and 32% respectively. The relative collection efficiencies of the Gelman 47 mm Membrane Filter (MF), PBI 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 due to 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 similar to that of the reference AGI-30, because the organism is an endospore-former and is more resistant to desiccation. For aerosols of free bacteria, the Andersen 6-stage impactor, the Ace Glass AGI-30, and the Andersen 1-stage impactor gave comparable results.

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Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

Introduction

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.

The Andersen Six-Stage Viable (Microbial) Particle Sizing Sampler and the Ace Glass All-Glass Impinger-30 have been regarded as the samplers of choice for the enumeration of viable microorganisms.[1,2,3,4] Several studies have compared currently available bioaerosol samplers.[5,6,7,8,9,10,11] However, the majority of these comparison studies are based on side-by-side sampling conducted under field conditions. Due to 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.

Materials and Methods

Aerosol Samplers

The following samplers were evaluated: the 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 Bionalyse Italiana (PBI) Surface Air System Sampler (SAS, Pool Bionalyse Italiana, Milano, Italy), Mattson-Garvin Slit-to-Agar Air Sampler (STA, Barramundi Corporation, formerly Mattson-Garvin Company, Homosassa Springs, FL), Biotest Reuter Centrifugal Air Sampler (RCS, Biotest Diagnostics Corporation, Denville, NJ), Gelman 47 millimeter (mm) Membrane Filter Air Sampler (MF, Gelman Sciences Incorporated, Ann Arbor, MI), and Ace Glass All-Glass Impinger-30 Sampler (AGI-30, Ace Glass Incorporated, Vineland, NJ).

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 liters per minute (lpm). Particle velocity increases as the air flows through successively smaller holes. Large particles, ≥ 7 micrometers (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.[12]

As a practical matter, these impactors 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 the mass of the particles larger than the cut-diameter (d_{50}) that gets through the impactor equals the mass of particles below the d_{50} that are collected. Hence, the d_{50} is the aerodynamic diameter above which the collection efficiency of the impactor approaches 100%.[13] Figure 1 shows the cut-diameter (d_{50}) of each stage of the Andersen 6-STG sampler.

Sampling plates were prepared by pouring 45 ml of Bacto Tryptic Soy Agar (TSA, DIFCO Laboratories, Detroit, MI) aseptically into each of the six 100 mm x 15 mm sterile plastic Petri plates so that the gap between the sieve and agar surface met the manufacturer's specification.[14,15] All inside surfaces were maintained sterile until sampling. After sampling for approximately 180 seconds (s) or the equivalent of 84.9 l (liters), the plates were removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 hours (h).

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 lpm. Figure 1 shows the cut-diameter (d_{50}) of each stage of the sampler. The two sampling plates were each prepared aseptically with 20 ml of TSA so that the gap between the sieve and agar surface met the manufacturer's specification.[14] After sampling for approximately 180 s or the equivalent of 84.9 l, the plates were removed from the sampler, covered, inverted, and incubated at a temperature of 35°C, and enumerated after 24 h.

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 lpm. Figure 1 shows the

cut-diameter (d_{50}) of the sixth stage of the sampler as 0.65 μm. The plate was poured aseptically using 45 ml of TSA so that the gap between the sieve and agar surface met the manufacturer's specifications.[15] After sampling for approximately 180 s or the equivalent of 84.9 l, the plate was removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

Pool Bionalyse Italiana Surface Air System Sampler

The PBI SAS sampler operates similarly in principle to the Andersen 1-STG sampler; however, the impactor stage consists of 219 holes, drawing air at a flow rate of 90 lpm. The holes are approximately the same diameter as the second stage of the Andersen 6-STG sampler. Figure 1 shows the cut-diameter (d_{50}) of the SAS sampler as approximately 2 μm. The SAS sampler has an internal timer and can be set to sample from zero to five minutes, in 20 second increments. A 50 mm Replicate Organism Direct Agar Contact (RODAC) plate was prepared aseptically with TSA so that a convex meniscus formed at the rim of the plate.[9] After sampling for approximately 300 s or the equivalent of 450 l, the plate was removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

Mattson-Garvin Slit-to-Agar Air Sampler

The STA sampler uses inertial impaction to collect viable organisms onto a culture medium at a flow rate of 28.3 lpm. The sampler has a tapered slit which produces a rectangular jet stream towards the surface of a rotating Petri plate, when air is sampled. Figure 1 shows the cut-diameter (d_{50}) of the STA sampler as approximately 0.5 μm. A 150 mm x 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.[16] After sampling for approximately 180 s 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 h.

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 theoretical flow rate of the sampler is 280 lpm; however, the flow rate was experimentally determined to be approximately 210 lpm by Macher and First.[17,18] 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 has an area of approximately one square centimeter. Figure 1 shows the cut-diameter (d_{50}) of the RCS sampler as

approximately 4 μm. After sampling for approximately 480 s or the equivalent of 1680 - 2240 l, the strip was removed from the sampler, covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

Gelman 47 mm Membrane Filter Air Sampler

The MF sampler is widely used for aerosol sampling due to its low cost and simplicity of operation. This air filtration apparatus consists of an aluminum 47 mm open-faced filter holder with a sterile 47 mm membrane filter made of cellose 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 s at a flow rate of 5 lpm, 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 was covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

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 cut-diameter (d_{50}) of the AGI-30 sampler as approximately 0.3 μm. The AGI-30 sampler operates by

drawing aerosols at a flow rate of 12.3-12.6 lpm through an inlet tube curved to simulate the nasal passage.[20] In contrast to impaction of bioaerosols onto agar, impingers may give higher bacterial counts in environments where bacteria are carried as aggregates, due mainly to th fact that bacterial clusters are broken up.[21] After sampling for approximately 360 s 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.[22] The membrane filter was placed in a 50 mm Petri plate on a pad saturated with TSB. The plate with the MF was covered, inverted, incubated at a temperature of 35°C, and enumerated after 24 h.

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).[23] The flow rate through these sampling instruments was verified to be within manufacturers' specification before each day of sampling 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. Sampling rate was found to be within 10%

of the manufacturer's specification (90 lpm). The RCS is not easy to calibrate or even verify the flow rate.[18] We followed the manufacturer's recommendation by using the Calibration-Set (Biotest) to check and if necessary correct the impeller blade angle. In addition, the internal timer was verified to within manufacturer's specification (8-minutes ± 2%) using 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.[24] The chamber was a stainless steel 0.61 m square duct, approximately 3.7 m long. Two baffles were located down stream of the aerosol inlet to promote uniform mixing of the aerosol with the flow stream. 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 for easy access to the inside of the chamber to place and remove samplers, and allowed application of chemical disinfectants. A schematic diagram of this Bioaerosol Chamber is shown in Figure 2.

The flow rate of air though the chamber was adjustable, but for the purpose of this study, the flow rate was maintained at 11.3 m^3/min.

This flow rate resulted in an air velocity of approximately 0.54 m/sec. Vertical and horizontal velocity traverses were performed 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 mono-disperse aerosol of oleic acid was generated using a Berglund-Liu Vibrating Orifice Monodisperse Aerosol Generator.[25,26] An aerosol with an aerodynamic diameter 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 using a TSI® Aerodynamic Particle Sizer (APS, TSI Incorporated, St. Paul, MN) and found to be 5.37 μm ± 1.13 μm. The mass concentration for these traverses was measured to be 0.126 mg/m^3 ± 0.035 mg/m^3.

Preparation of pure culture suspensions

Cultures used included E. coli (ATCC 11229) and B. subtilis (ATCC 6633, DIFCO). E. coli is a Gram negative, non-spore forming bacteria which is approximately 0.5 μm wide and 2.0 μm long. B. subtilis is a Gram positive, endospore-forming bacteria of similar size and shape. Active E. coli and B. subtilis cultures were inoculated into approximately

100 ml of TSB and incubated for 18-24 hours 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, NJ), capped, and centrifuged at 1500 g for 10 minutes in an IEC CENTRA-4B® General-Purpose Centrifuge (International Equipment Company, Needham, MA). 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. The cell concentration was determined by turbidimetric measurement of this suspension. The transmittance of monochromatic light at a wavelength of 450 nanometers through the cell suspension was measured using a Spectronic® 20 spectrophotometer (Milton Roy Company, formerly Bausch & Lomb, Rochester, NY). The concentration of cells was estimated from a previously developed calibration curve 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.[27] A dilution series was performed for standard plate count enumeration.[28] The nebulizer then generated an aerosol of mostly single bacteria cells with a chamber concentration of approximately 2000 CFU/m^3.

Aerosol generation

A Collison nebulizer was used to aerosolize the suspension of E. coli cells. The BGI 6-jet modified MRE-type Collison Nebulizer (BGI Collis Nebulizer, BGI Incorporated, Waltham, MA) was used in this study. Within the nebulizer, compressed air expanded from a pressure of 140 kilopascals (kPa) at the stem into the six side jets. The reduction of static pressure forced water up the tube at the bottom of the stem, similar to an eductor. This fluid 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 wall of the glass vessel; however, the minute amount of liquid which escaped impact comprised only the finest tail of the drop-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 seconds; where as a 2.9 μm water droplet would have had a wet lifetime of 0.03 seconds. The wet lifetime is proportional to diameter squared at a given temperature and relative humidity.[29] 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.[30] Theoretically, mainly bacterial cells make their way to the sampler location in the aerosol chamber, a transit time of approximately six seconds or approximately 10 life times for a

10 μm water droplet. To validate the assumption that only particles containing either one bacteria cell and water, or just water, particles from the Collison nebulizer were collected on a glass slide and viewed using a phase contrast microscope (LABOPHOT-2, Nikon Corporation, Tokyo, Japan). No clumping of bacteria was 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 indicates that a majority of the particles collected had an d_{ae} were 1.1 and 2.1 μm.

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 (five minutes on and five minutes off for the first 90 minutes) and aliquots of the nebulized solution were diluted and plated. In parallel, aliquots of the suspension (static cells) were also diluted and plated. As shown in Figure 4 and Figure 5, 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 five hours 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 h at a temperature of 35°C in a Forma Scienti c Water Jacketed Incubator Model 3158 (Forma Scientific, Marrietta, OH , all plates were enumerated using a New Brunswick Model C-110W Colc Counter (New Brunswick Scientific Co., Inc., Edison, NJ). All CFUs on each plate were counted, which included a number of "multiple hits". Because it was very easy to identify more than one CFU at an impaction point, we did <u>not</u> apply a positive hole statistical adjustment. Detailed discussions of the positive hole statistical adjustment are found in the literature.[12,31,32]

Experimental Design

The eight microbial samplers were compared to an AGI-30 sampler located approximately 0.6 meters upstream of the 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 and/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 the AGI-30 has a calculated d_{50} of 0.30 μm. Four samplers were tested during each sampling session for a total of

10 sessions, with only one sampling session per sampling day. The order in which the samplers were tested was randomized. Samples were taken with four replications.

Results

Overall evaluation of eight bioaerosol samplers

All statistical analyses were performed using SAS Version 6.03.[33] 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), all 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 "SAMPRATIO." Analysis of Variance (ANOVA) tables were generated using the General Linear Models Procedure (PROC GLM). The dependent variable, SAMPRATIO, was regressed with three independent variables: sampler type; day of evaluation; and sequence within each day. The means and the 95% confidence intervals for the concentration ratios are shown in Figure 6 and Figure 7. The results of the multiple comparison tests of SAMPRATIO for each sampler are shown in Table I and Table II. 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.

Recovery of vegetative cells vs. bacterial endospores

Data shown in Figure 6 and Figure 7 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 endospores was observed in the performance of the MF sampler. The low relative efficiency of the MF with E. coli was likely due to 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 confirms other reports in the literature.[5,6,34,35,36] 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 due to 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, however, tends to cause the destruction of some vegetative cells or may result in overestimation due to the dispersion of dust particles and the breaking up of clumps of bacteria.[35,36,37]

The Mattson-Garvin STA relative sampling efficiency was marginally lower than the others. Because of the low d_{50} (0.5μm), the STA should have a relative sampling efficiency similar to the 6-STG, 1-STG, and AGI-30.

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. By superimposing Figure 1 on Figure 3, it is clear that the lower tail of the aerosol distribution is below the d_{50} 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 (< 2μm) determined by Mach r and First and the theoretical efficiency for small particles calcula ed using the empirical formula developed by Biotest indicate the poor collection characteristics of this instrument. With a d_{50} of 3.8 for the RCS and a d_{50} of 2 μm for the SAS, few if any free bacteria w d impact on the agar surface.[17,18] The advantage of portability of these two instruments does not out weigh the inefficiency of these instruments to sample free bacteria. These samplers have shown better recovery of larger particles.[9,18]

The vegetative cells of E. coli were essentially killed by desiccation using the Gelman MF sampler while 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.[38,39]

The results of this study indicate that the Andersen 6-STG, Andersen 1-STG, and Ace Glass AGI-30 samplers are 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.[2,3,4] Recently, the Andersen 1-STG has been suggested by the American Conference of Governmental Industrial Hygienists Committee on Bioaerosols as a sampler of choice.[3] 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.

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Table Captions.

Table I. Bonferroni (Dunn) *t* tests of the variable SAMPRATIO for eight bioaerosol samplers challenged with an aerosol of Escherichia coli.

Table II. Bonferroni (Dunn) *t* tests of the variable SAMPRATIO for eight bioaerosol samplers challenged with an aerosol of Bacillus subtilis.

Table I. Bonferroni (Dunn) *t* tests of the variable SAMPRATIO for eight bioaerosol samplers challenged with an aerosol of Escherichia coli.

Bonferroni Grouping		Mean	N	Sampler
	A	107.1	20	6-STG
	A			
B	A	100.1	20	AGI-30
B				
B	C	93.4	20	1-STG
	C			
	C	88.7	20	STA
	D	68.1	20	2-STG
	E	0.089	20	SAS
	E			
	E	0.028	20	RCS
	E			
	E	< 0.001	20	MF

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

83

Table II. Bonferroni (Dunn) *t* tests of the variable SAMPRATIO for eight bioaerosol samplers challenged with an aerosol of Bacillus subtilis.

Bonferroni Grouping		Mean	N	Sampler
	A	105.9	20	6-STG
	A			
B	A	101.4	20	AGI-30
B				
B	C	96.6	20	MF
B	C			
B	C	92.6	20	1-STG
	C			
	C	90.7	20	STA
	D	66.7	20	2-STG
	E	2.59	20	SAS
	E			
	E	< 0.001	20	RCS

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

Figure Captions.

FIGURE 1. Cut-off diameters (d_{50}) for the seven bioaerosol samplers th operate on the principle of impaction.

FIGURE 2. Schematic of the bioaerosol chamber.

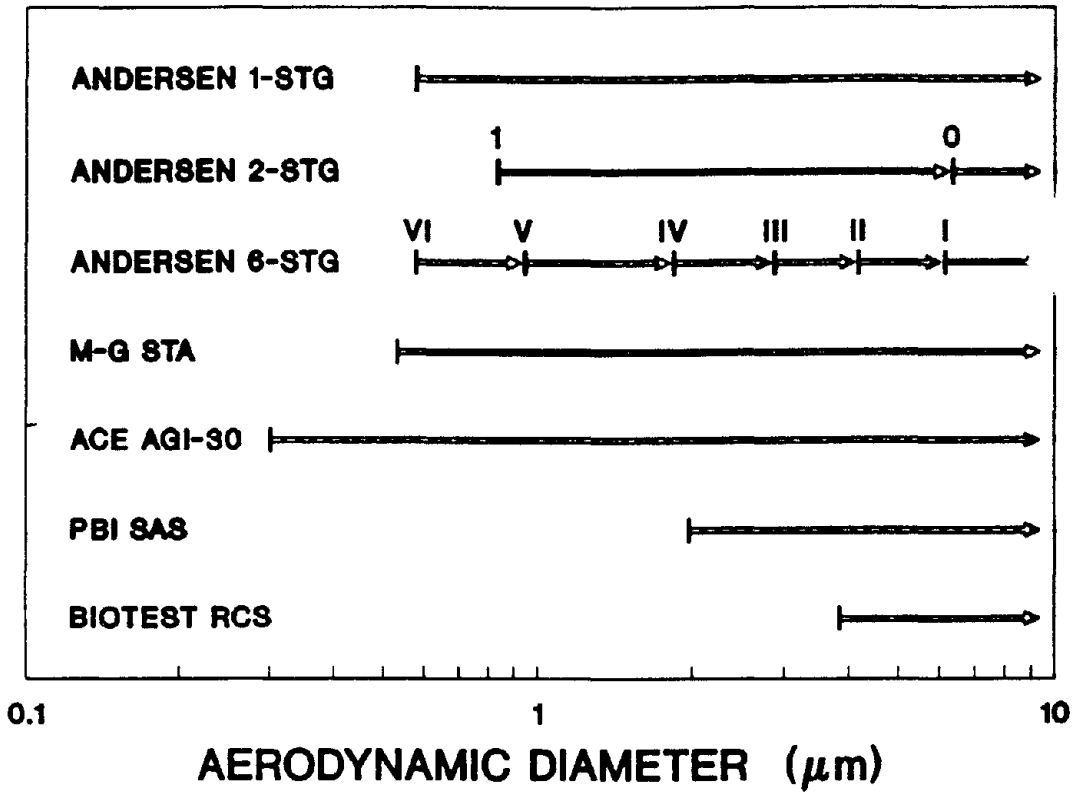
FIGURE 3. Size distribution of E. coli and B. subtilis aerosols, collected on an Andersen 6-STG sampler.

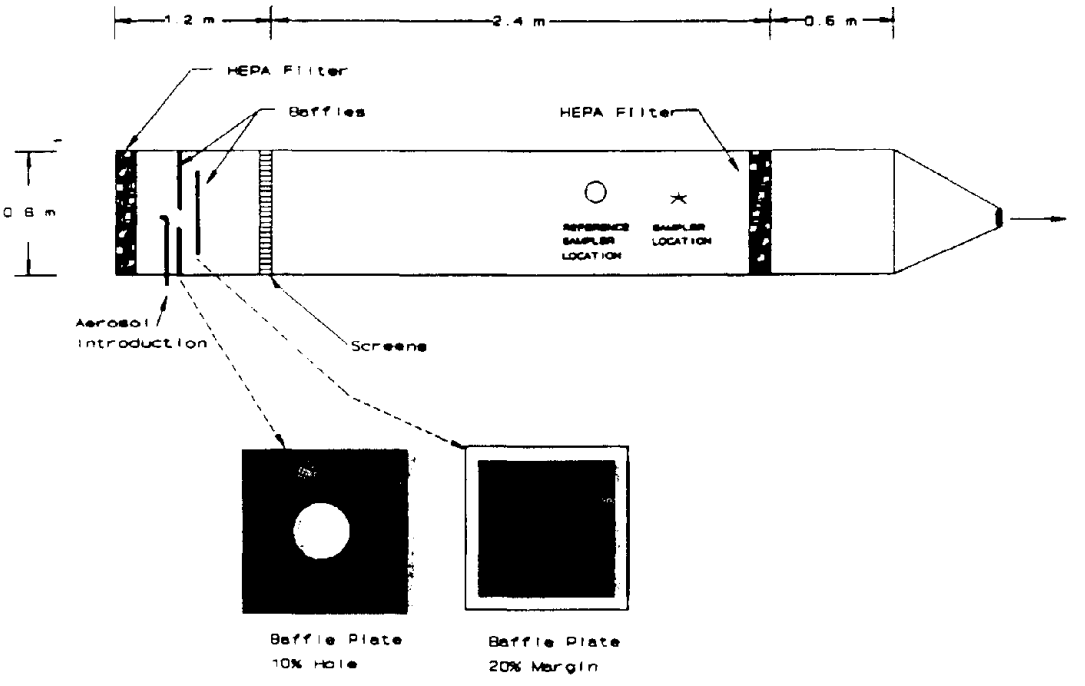
FIGURE 4. Comparison of the viability of E. coli in phosphate-buffered dilution water when nebulized and when in a static environment.

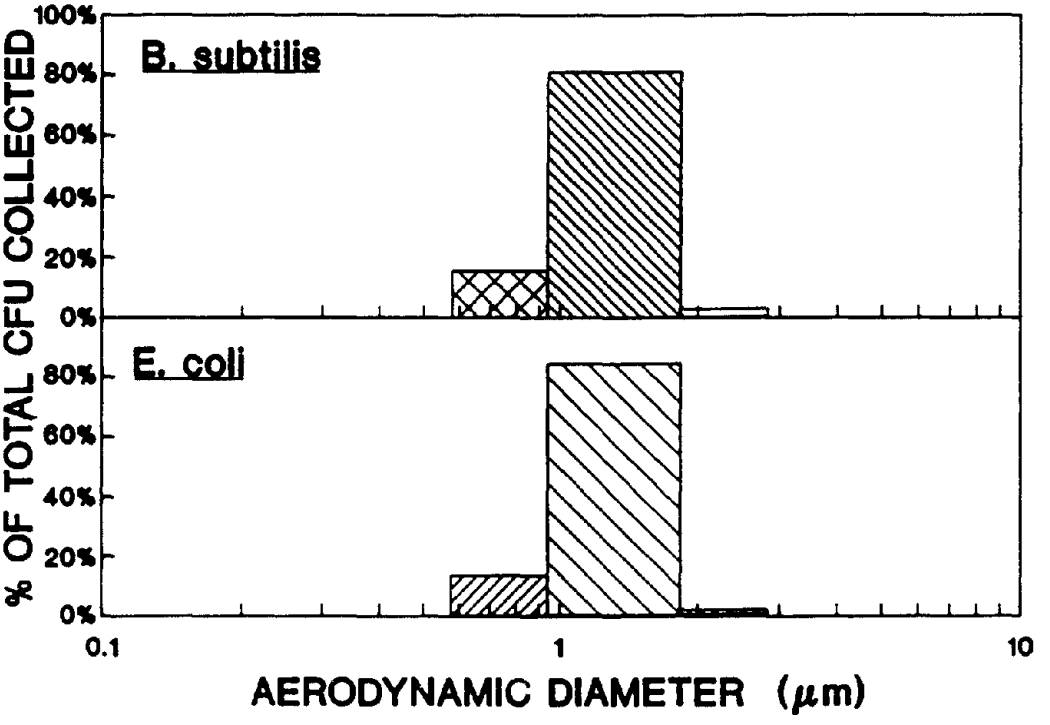
FIGURE 5. Comparison of the viability of B. subtilis in phosphate-buffered dilution water when nebulized and when in a static environment.

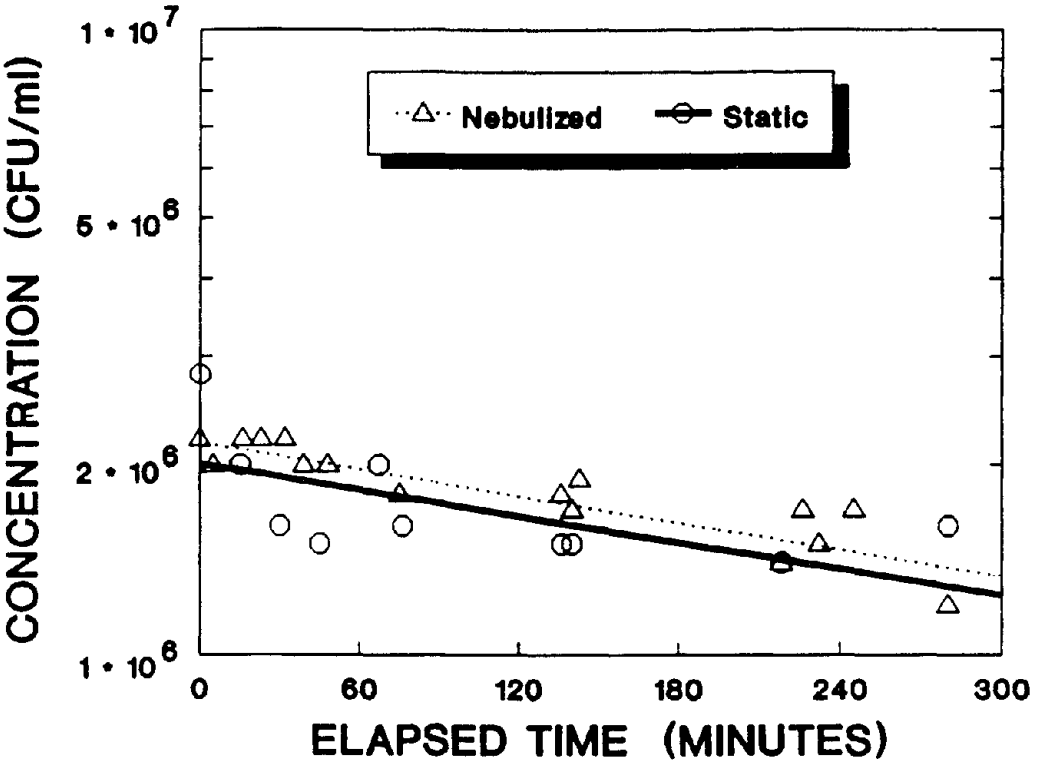
FIGURE 6. Recovery of aerosols of E. coli using eight bioaerosol samplers. Arithmetic mean of the concentration ratio of the test sampler to the control sampler, in percent, and the 95% confidence limits about the mean.

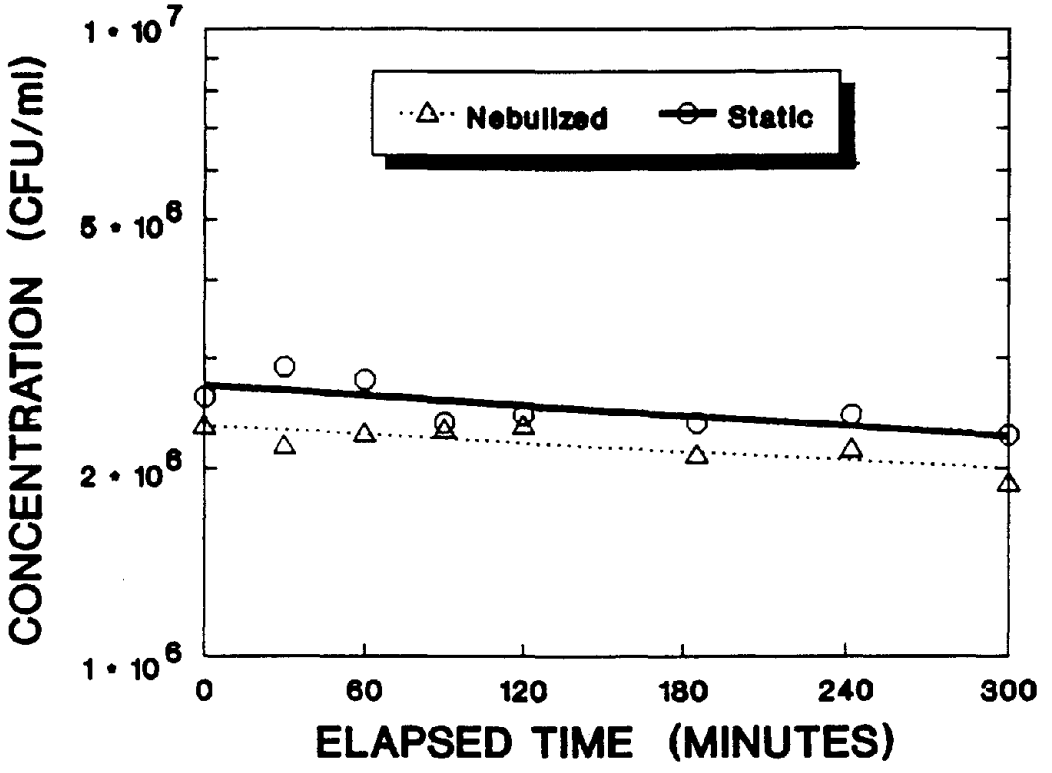
FIGURE 7. Recovery of aerosols of B. subtilis using eight bioaerosol samplers. Arithmetic mean of the concentration ratio of the test sampler to the control sampler, in percent, and the 95% confidence limits about the mean.


ANDERSEN 1-STG
ANDERSEN 2-STG
1
0
ANDERSEN 6-STG
VI
V
IV
III
II
I
M-G STA
ACE AGI-30
PBI SAS
BIOTEST RCS
0.1
1
10
AERODYNAMIC DIAMETER (μm)




B. subtilis
E. coli
100%
80%
60%
40%
20%
0%
% OF TOTAL CFU COLLECTED
0.1
1
10
AERODYNAMIC DIAMETER (μm)


CONCENTRATION (CFU/ml)
1 • 10^7
5 • 10^6
2 • 10^6
1 • 10^6
Nebulized
Static
0
60
120
180
240
300
ELAPSED TIME (MINUTES)


CONCENTRATION (CFU/ml)
1 • 10^7
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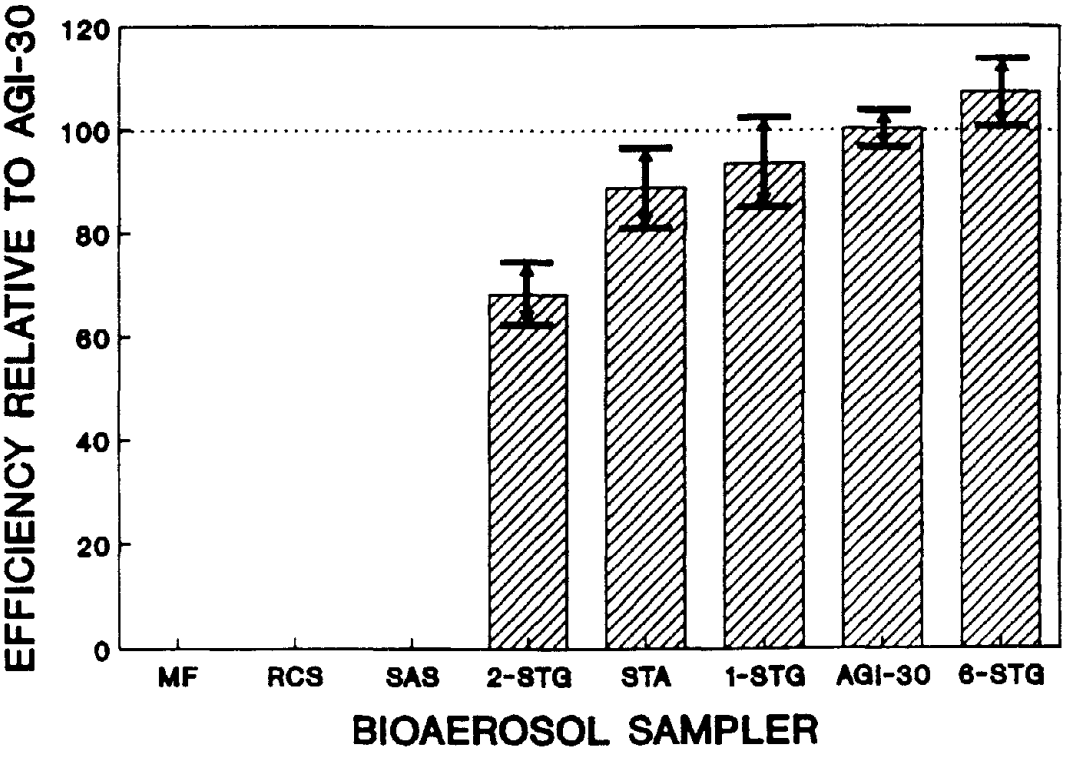
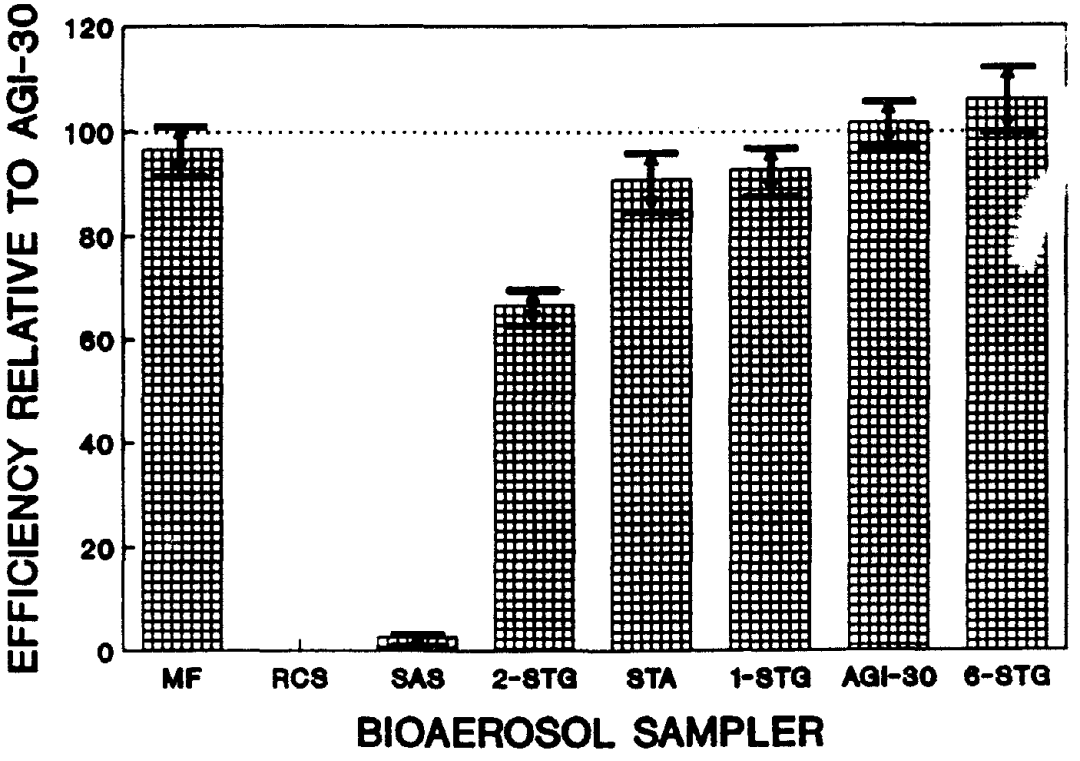
Nebulized
Static
0
60
120
180
240
300
ELAPSED TIME (MINUTES)


EFFICIENCY RELATIVE TO AGI-30
120
100
80
60
40
20
0
MF
RCS
SAS
2-STG
STA
1-STG
AGI-30
6-STG
BIOAEROSOL SAMPLER


EFFICIENCY RELATIVE TO AGI-30
120
100
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60
40
20
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MF
RCS
SAS
2-STG
STA
1-STG
AGI-30
6-STG
BIOAEROSOL SAMPLER

Design and evaluation of an aerosol chamber for testing of aerosol samplers

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Disclaimer

Mention of commercial names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

ABSTRACT

An aerosol test chamber was designed and built by NIOSH researchers to evaluate aerosol samplers. A simple design was utilized, was easy to construct, and is similar in design to the ASHRAE Air Cleaner Standard Test Duct used for rating air cleaners. The flow rate through the chamber could be varied by simply adjusting a damper. The chamber was fabricated of stainless steel; to allow sampling for both microbiological and physical aerosols. The chamber is 0.61 m on each side and is 3.7 m long. The chamber supply and exhaust air are purified with HEPA filters. Uniform velocity and particle concentration distributions were determined for a single baffle and a two baffle design. For the latter, two baffles, the first with a 10% open area in the center and the second with a 20% opening along the walls of the chamber, were located downstream of the point of aerosol introduction (the aerosol entered into the chamber flow of oncoming air). Air, with the entrained aerosol, is accelerated though the center of the first baffle opening, around the second baffle, which then redirects the air toward the sides of the chamber. This design, versus the single baffle (a 20% opening along the walls of the chamber) design, was used to improve the particle concentration uniformity and the consistency of the velocity profile across the chamber face. A vibrating orifice monodisperse aerosol generator was used to produce aerosol particles of oleic acid with a nominal aerodynamic diameter of 10 μm (single baffle) and 5 μm (two baffles). The mass median aerodynamic diameter (MMAD) for the horizontal and vertical traverses for the single baffle chamber was measured to be 10.16 μm ± 0.12 μm and the particle number concentration was measured to be 0.197 part/cm^3 ± 0.064 part/cm^3. The MMAD for the horizontal and vertical traverses for the two two baffle chamber was measured to be 5.37 μm ± 0.08 μm and the mass concentration was measured to be 0.217 part/cm^3 ± 0.01 part/cm^3. For the single baffle, a flow rate of 340 m^3/hr resulted

in an air velocity of 0.20 m/sec ± 0.06 m/sec. For the two baffle, a flow rate of 340 m^3/hr resulted in an air velocity of 0.26 m/sec ± 0.01 m/sec and a flow rate of 680 m^3/hr resulted in an air velocity of 0.57 m/sec ± 0.01 m/sec. The "stabalization" of the velocity profile and the improved uniformity of the particle concentration across the cross-sectional area of the chamber demonstrate the effectiveness of the second baffle and the re-direction of the aerosol entry point.

BACKGROUND

Occupational exposures to microorganisms and their metabolic byproducts have recently become issues of increasing awareness in the biotechnology industry, agricultural environments, and indoor air quality investigations.[1,2,3,4,5] Sampling methods for the assessment of microbial aerosols in the ambient environment have been well established.[6,7,8] There are a number of documented studies evaluating the comparability of currently available bioaerosol samplers including the three Andersen cascade impactors, the Biotest RCS sampler, the Pool Bionalyse Italiana SAS sampler, the slit-to-agar impactor, the all glass impinger, and the membrane filter sampler.[9,10,11,12,13,14,15] However, the results of the majority of these comparison studies are based on side-by-side sampling extracted under field conditions. Due to 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. Evaluation and calibration of sampling instruments under controlled experimental conditions promotes an increased awareness of the sampler capabilities and enhances the degree of confidence which is imparted to the sampling results. Because of the limited documentation of environmentally controlled sampler assessments, NIOSH researchers evaluated several bioaerosol samplers in an aerosol chamber under controlled conditions. The samplers were challenged with (1) monodisperse particles of oleic acid tagged with a fluorescent dye and (2) aerosolized single cells of *Escherichia coli* and *Bacillus subtilis*.[16,17] This paper will focus on the design and testing of the aerosol chamber used to evaluate these bioaerosol samplers.

AEROSOL CHAMBER DESCRIPTION

The original chamber, with a single baffle, was constructed of stainless steel with a duct length of approximately 3.7 m and a square cross section of 0.61 m. It was designed with the flexibility to allow modifications that were required during the use of microbial aerosols. The following specific features were incorporated into the original chamber design (OCD):

* Stainless steel construction to facilitate cleaning, disinfecting, and minimization of corrosion;
* Baffle plate near the aerosol inlet (with a 20% open area margin at the chamber walls) to aid in the distribution of the aerosol in the chamber;
* High efficiency particulate air (HEPA) filters at the inlet to provide clean air to the chamber and at the outlet to ensure no contamination outside of the chamber;
* Variable flow rate by an adjusting a damper;
* An observation and single access ports (30 x 60 cm Plexiglass® windows held in place by quick-action toggle clamps).

After initial evaluation of the chamber, enhancements were added to provide a more uniform distribution of aerosol particles across the chamber cross-section at the point of sampler evaluation (the sampling plane). These enhancements included the redirection of the introduced aerosol into the flow of incoming air and the addition of a baffle having a circular, central opening (10% of the cross-sectional area) between the aerosol inlet and a second baffle having a square, marginal opening (20% of the cross-sectional area) as shown in Figure 1. The air, with the entrained aerosol, is accelerated

through the center of the first baffle, around the second baffle, and through the flow straightener. Three fused-silica windows were installed in the top of the chamber (to permit sterilization by UV radiation) along with 3 additional observation and access ports on the sides of the chamber. In addition, the air was exhausted to the roof of the building. A schematic of the modified chamber design (MCD) is shown in Figure 2. For coordinate orientation, the x-axis is defined along the cross-section of the chamber floor, the y-axis is defined along the cross-section of the chamber side walls, and the z-axis is defined along the centerline of the chamber. The aerosol chamber was designed to give an air velocity at the point of the sampler challenge test of approximately 25-50 cm/sec resulting in a volumetric flow rate of 340-680 m^3/hr. This low velocity range falls within the still-air sampling criteria (reminiscent of real-world conditions) is defined by the following equation:[18]

$$U_0 \leq \frac{1}{5}\left(\frac{Q}{4\pi\tau^2}\right)^{1/4}$$

where U_0 is the maximum air velocity for which the still-air sampling criteria can be used, in cm/sec, Q is the sample flow rate in cm^3/sec, and τ is the relaxation time for a given particle diameter in sec. For 10 μm diameter particles (unit density sphere) at a sampling flow rate of 28 lpm, the maximum allowable air velocity in the chamber to meet the criteria is approximately 145 cm/sec.

Unlike the aerosol test chamber design by Marple and Rubow, this chamber is oriented on a horizontal axis.[19] This orientation facilitates placement of the chamber in the laboratory and access to its various parts. In many respects, the chamber is similar in design to the American Society for Heating, Refrigerating, and Air-conditioning Engineers air cleaner standard test duct.[20] Elutriation, which is an inevitable

consequence of the decay (due to gravity) of an aerosol particle trajectory along the critical path of a horizontal duct was not a problem for the specific particle sizes used in the bioaerosol sampler studies (up to 10 μm). The height, H in cm, that a particle will settle over the chamber length from the baffle plate to the sampler location, L_s in cm, is given by the equation:[18]

$$H = \frac{L_s V_{ts}}{V_{chamber}}$$

where V_{ts} is the terminal settling velocity at a given particle size in cm/sec and $V_{chamber}$ is the air velocity in the chamber in cm/sec. Based on this equation, the largest particle size used (10 μm) will settle 3.42 cm at the lower flow rate and 1.71 cm for the higher flow rate.

The Reynolds numbers (Re) corresponding to the air flows inside the chamber were determined from the following equation:[21]

$$Re = \frac{\rho l u}{\mu}$$

where u is the velocity of the chamber in cm/sec, l is the width of the chamber in cm, ρ is the density of the air in gm/cm, and μ is the viscosity of the air in gm/(cm-sec).

For the designed chamber velocities, 25 to 50 cm/sec, the Re were detemined to be in the turbulent regime (10,200 to 20,400 where greater than 4000 is turbulent).

EVALUATION OF CHAMBER

Horizontal and vertical traverses were conducted with a thermoanemometer (KURZ

1440, Kurz Instruments, Inc., Monterey, CA) to determine the velocity profile at the sampler location. For the OCD, a flow rate of 340 m^3/hr resulted in an air velocity of 0.20 m/sec ± 0.06 m/sec. For the MCD, a flow rate of 340 m^3/hr resulted in an air velocity of 0.26 m/sec ± 0.01 m/sec and a flow rate of 680 m^3/hr resulted in an air velocity of 0.57 m/sec ± 0.01 m/sec. Figure 3 presents the results of horizontal and vertical velocity traverses, for the OCD, at distances of 1.01 m and 2.43 m downstream from the second baffle. Note that each traverse for the OCD only extends to the center of the chamber. At 1.01 m downstream of the baffle (second baffle in Figure 2), the velocity profiles exhibit half of an unstable "frisbee" shape with the highest velocities occurring at the walls of the chamber and lower velocities (or velocity stabilization) occurring at the center of the chamber. The higher velocities at the chamber walls were primarily the result of the air "jetting" around the 20% margin of the second baffle. At 2.43 m downstream from the flow straightener, the velocity profiles took on a flatter appearance (again, the highest velocities occurring at the chamber walls). The stabilized velocities, in the center of the chamber, approached the desired chamber air velocity of 0.25 m/sec.

Observation of Figure 4 (horizontal and vertical velocity profiles of the MCD at 0.25 and 0.50 m/sec) indicates no significant change in the profiles. The consistent profiles of the MCD as compared to the OCD demonstrates the effectiveness of the chamber modifications in providing a uniform velocity profile along the horizontal and vertical axes.

The aerosol chamber was evaluated twice to determine particle distribution and velocity profile uniformity; specifically, first for the OCD and second for the MCD. A vibrating orifice monodisperse aerosol generator (VOMAG) was used to produce aerosol particles of oleic acid with a nominal aerodynamic diameter of 10 μm for the OCD and

5 μm for the MCD.[22] The VOMAG operates through the combined contributions of four parts: the liquid feed system, the droplet generator, the droplet dispersion system, and the aerosol flow system. The liquid feed system forces a liquid (consisting of a mixture of alcohol and oleic acid) through a membrane filter into the droplet generator at a constant rate. The droplet generator houses the orifice disc through which the liquid flows. An AC voltage is applied to the piezoelectric ceramic which vibrates the disc and disturbs the liquid jet at a constant (selectable) frequency. The dispersion air from the droplet dispersion system creates a turbulent air stream which forces the enveloped droplet stream into a dispersed conical shape. The aerosol flow system uniformly disperses the droplets and allows the alcohol to volatilize leaving individual droplets of a specific, predetermined size. From Berglund and Liu [1973] the size of the individual particles (D_p) can be computed from the following formula:

$$D_p = C^{\frac{1}{3}} \left(\frac{6Q}{\pi f} \right)^{\frac{1}{3}}$$

where Q is the liquid feed rate in cubic centimeters per minute, f is the vibration frequency of the piezoelectric ceramic in Hertz, and C is the concentration of the nonvolatile solute dissolved in the volatile solvent. The diameters of particles generated by the VOMAG can be calculated, from the above equation, to less than a 1% error from the generator operating conditions. The stability of the generated aerosol has been determined to have a variation in concentration less than 3%.[23] Due to the importance of keeping the orifice disc clear of particulate matter, all reagents used in this investigation were of spectroscopic grade. Microscopic analysis of the reagents however, revealed suspended particulates. To reduce clogging of the orifice disc, all solutions underwent filtration through a 0.45 μm pore size membrane filter. A series of static eliminators was placed around the base of the dilution column (approximately 5 cm

above the point of aerosolization) to neutralize the charge of the aerosol particles before entering the chamber. For the OCD, the aerosol entered from the floor of the chamber whereas for the MCD, the aerosol entered from the side. In addition, for the MCD, the aerosol was introduced approximately 1.5 cm short of the chamber centerline to account for the momentum of the particles in the lateral direction (i.e. perpendicular to the flow of oncoming chamber air).

Horizontal and vertical chamber traverses were conducted in the sample plane using an Aerodynamic Particle Sizer (APS 33B, TSI, Inc. Minneapolis, MN) which provided real-time detection of aerodynamic particle size and particle concentration. The APS accelerates aerosol particles through a nozzle which then pass through a laser velocimeter.[24,25,26] The inertia of the particles causes the particles to lag behind the downstream air from the nozzle. The extent of the lag between the particle velocity and the gas velocity is based on the aerodynamic diameter of the particle. Although the APS was calibrated with Polystyrene Latex spheres (solid particles), the effect of liquid droplet deformation in the sample air stream should not affect the uniformity of the particle concentrations of a specific diameter from location to location in the chamber.[27] The duration of each APS sampling period was 20 sec for the OCD and 60 sec for the MCD. The mass median aerodynamic diameter (MMAD) for the horizontal and vertical traverses for the OCD was measured to be 10.16 μm ± 0.12 μm and the particle number concentration was measured to be 0.197 part/cm^3 ± 0.064 part/cm^3. The MMAD for the horizontal and vertical traverses for the MCD was measured to be 5.37 μm ± 0.079 μm and the particle number concentration was measured to be 0.217 part/cm^3 ± 0.010 part/cm^3. These data are graphically presented in Figure 5 and Figure 6.

Observation of Figure 3 indicates a fairly stable particle concentration 15 cm to either side of the centerline of the chamber. There is some variation as the x-coordinate approaches the chamber walls but this may be the result of the increased turbulence at the walls and/or the higher velocity at the walls caused by the air "jetting" through the open area margin baffle (the second baffle in Figure 2).

Observation of Figure 4 indicates a dramatic rise in the mass concentration as the distance from the chamber floor increases. Consideration of the air flow characteristics of the chamber and the aerosol introduction inlet provides an explanation for this phenomenon. The aerosol from the monodisperse particle generation device enters the chamber with a flow perpendicular to the chamber air flow. The upward inertia carries the aerosol over the second baffle. Mixing of the aerosol and the chamber air occurs due to the turbulence created after the baffle, however, the uneven distribution of the aerosol in the baffle margins creates the increasing concentration gradient from the floor to the top of the chamber. This "explanation" is further validated by the observation of oleic acid residue on the chamber ceiling directly above the aerosol introduction inlet. Observation of the horizontal and vertical concentration profiles in Figure 7 and Figure 8 indicates no significant change in the horizontal and vertical particle number concentration profiles except at the walls of the chamber, approximately a 35% difference (between the velocities at the walls) in the horizontal concentration profile and 33% in the vertical concentration profile. The improvement (uniformity) of the vertical concentration profile of the MCD as compared to the OCD demonstrates the effectiveness of the chamber modifications in providing a uniform particle concentration along the horizontal and vertical (x and y) axes.

CONCLUSIONS

The use of viable and non-viable aerosol particles in a small enclosed space for the evaluation of bioaerosol samplers introduced a number of constraints. The aerosol chamber has to be capable of producing a constant velocity profile and a uniform particle distribution across the chamber cross-section at the point of sampler challenge. The air entering or exiting the chamber must be filtered to provide an uncontaminated flow of air in the sample space and to contain any aerosols generated in the interior. Lastly, the chamber must easily and conveniently lend itself to sterilization during the aerosolization of microorganisms.

Wind-tunnels have been widely accepted for the determination of fluid mechanics flow characteristic responses to obstacles placed in the path of fluid air streams.[21,28] Other "chambers", whose designs have application potential for the investigation of situations encountered in the industrial hygiene community, have been documented.[19,20,28] However, constraints, such as those encountered during the bioaerosol sampler evaluations, may require a chamber design that provides highly restrictive parameters (i.e. stable particle distributions and constant velocity profiles) and wide-applicability to various fields of aerosol study. The modified aerosol chamber described in this paper provides a stable particle mass concentration in the horizontal and vertical planes along with a relatively constant velocity profile. The experimental uses of this chamber to the present time included the determination of sampling efficiencies of bioaerosol samplers using artificially generated aerosol particles of oleic acid and single-cell microorganisms.[16,17] One disadvantage of the small chamber size is that it

limits the number of sampling instruments that can be evaluated concurrently during a single experimental run.

ACKNOWLEDGEMENTS

This work was supported, in part, by the U.S. Environmental Protection Agency, Office of Research and Development through Interagency Agreement DW75932393-01-2 with NIOSH. Special thanks to Robert Hughes, Dennis O'Brien, and Paul Baron, NIOSH, for assistance in the design of the chamber and to Daniel Watkins, NIOSH, for constructing and modifying the chamber.

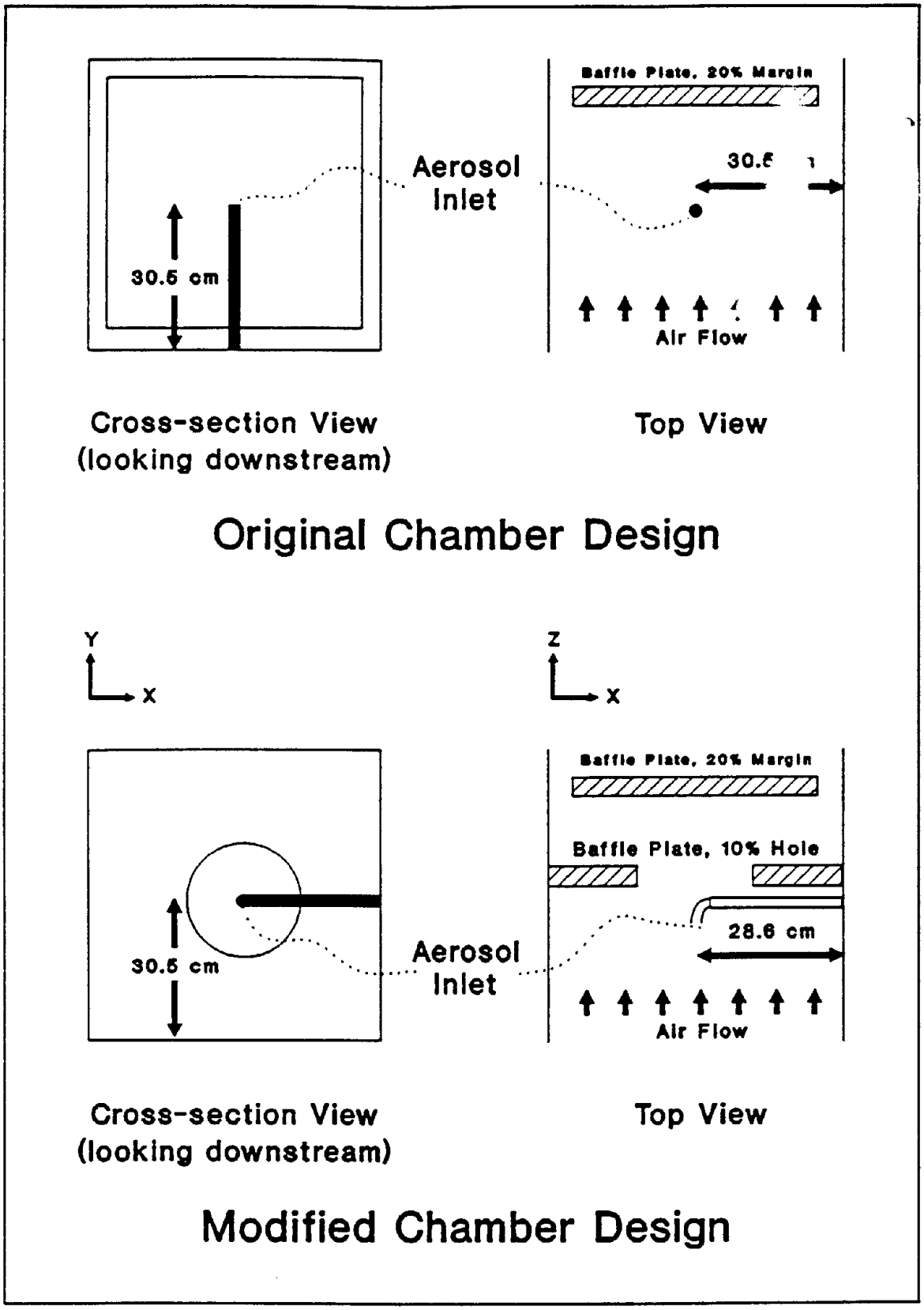

Figure 1. Original and modified aerosol inlet and baffle configurations.

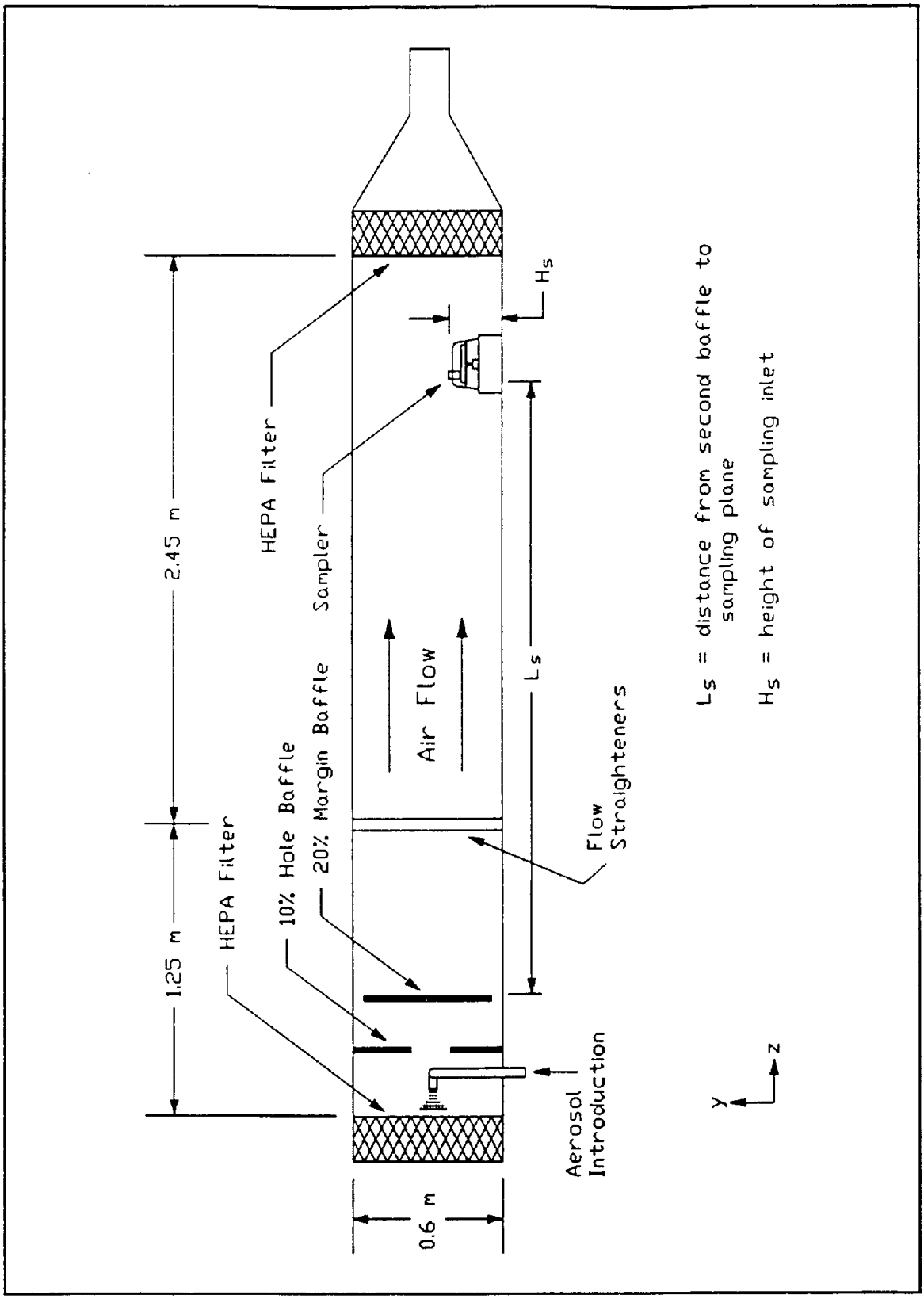

Figure 2. Aerosol Chamber (modified design)

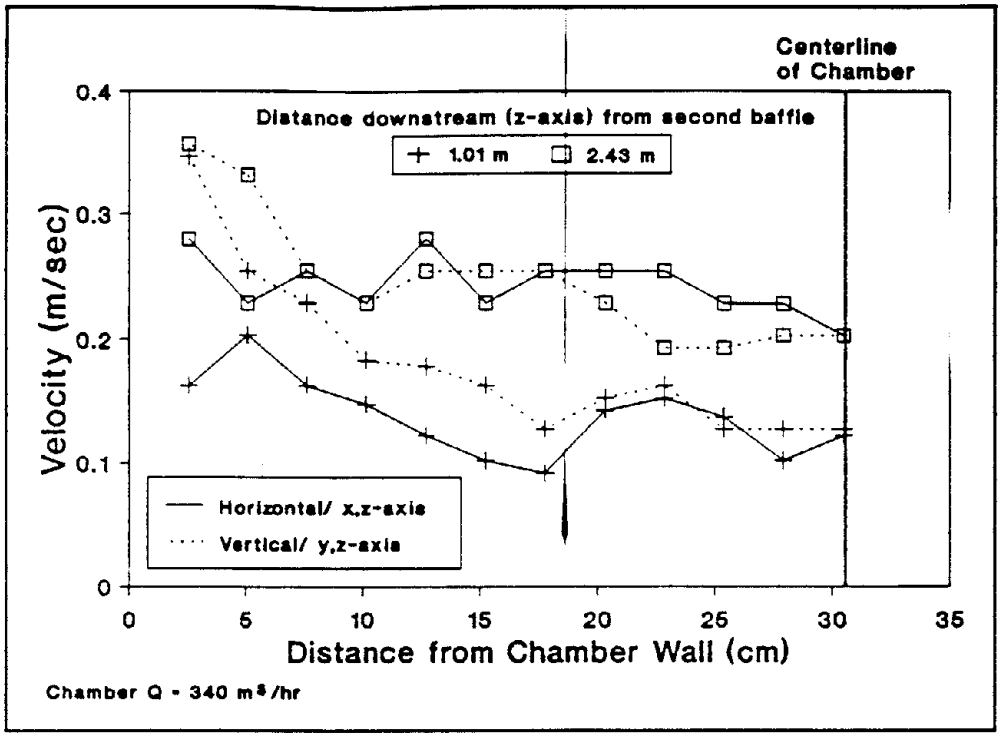

Figure 3. Velocity, horizontal (from the chamber side wall, x-axis) and vertical (from the chamber floor, y-axis) traverses for the OCD.

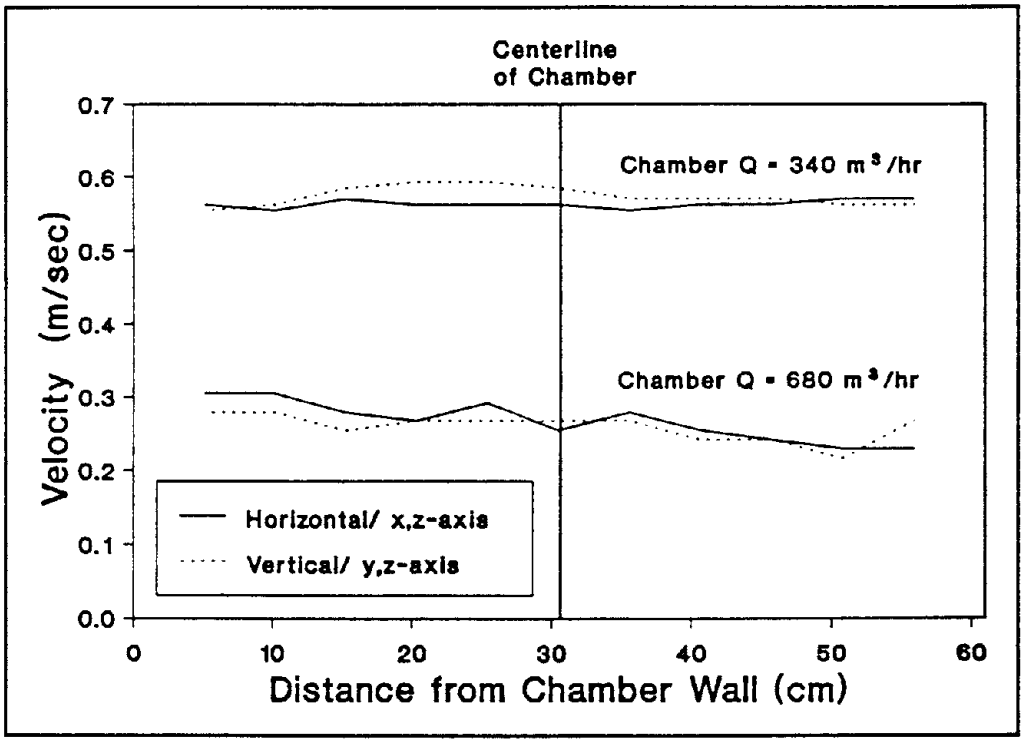

Figure 4. Velocity, horizontal (from the chamber side wall, x-axis) and vertical (from the chamber floor, y-axis) traverses for the MCD at 2.43 m downstream of the second baffle.

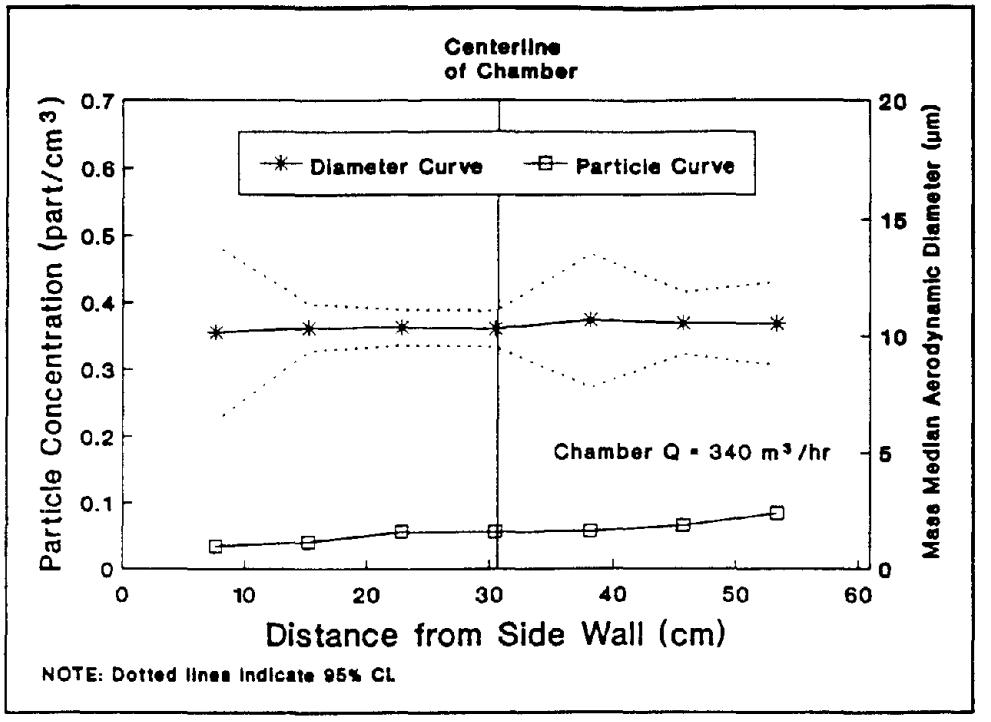

Figure 5.Original chamber design particle mass concentration and MMAD - horizontal traverse (2.79 m downstream, z-axis, from second baffle and 0.14 m from, x-axis, the chamber side wall).

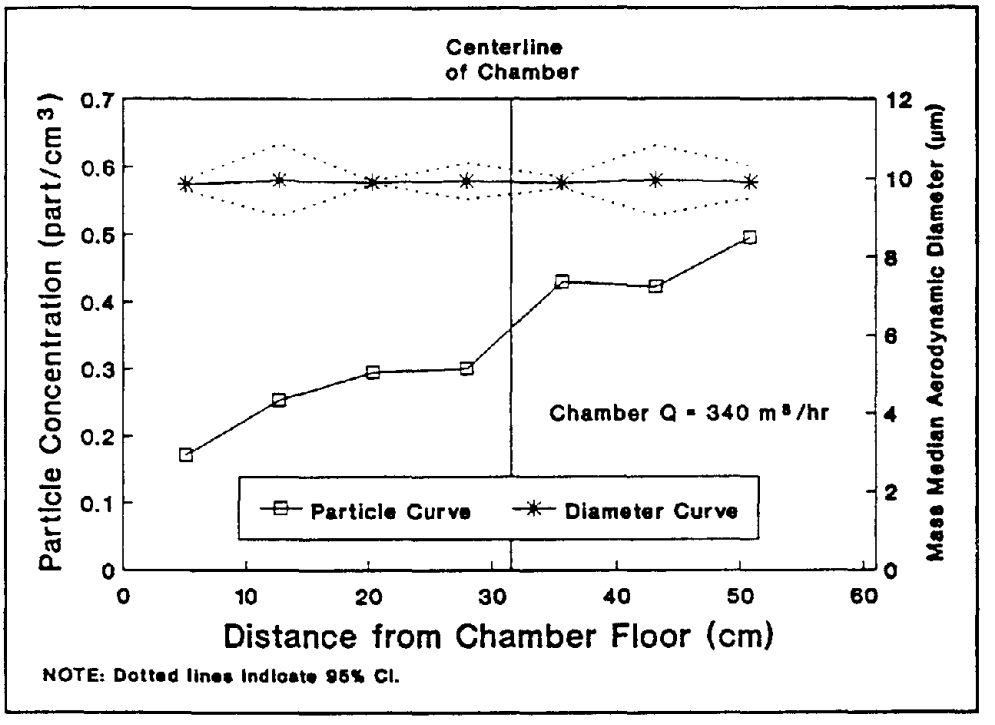

Figure 6.Original chamber design particle mass concentration and MMAD - vertical traverse (2.79 m downstream, z-axis, from second baffle and 0.30 m above, y-axis, the chamber floor).

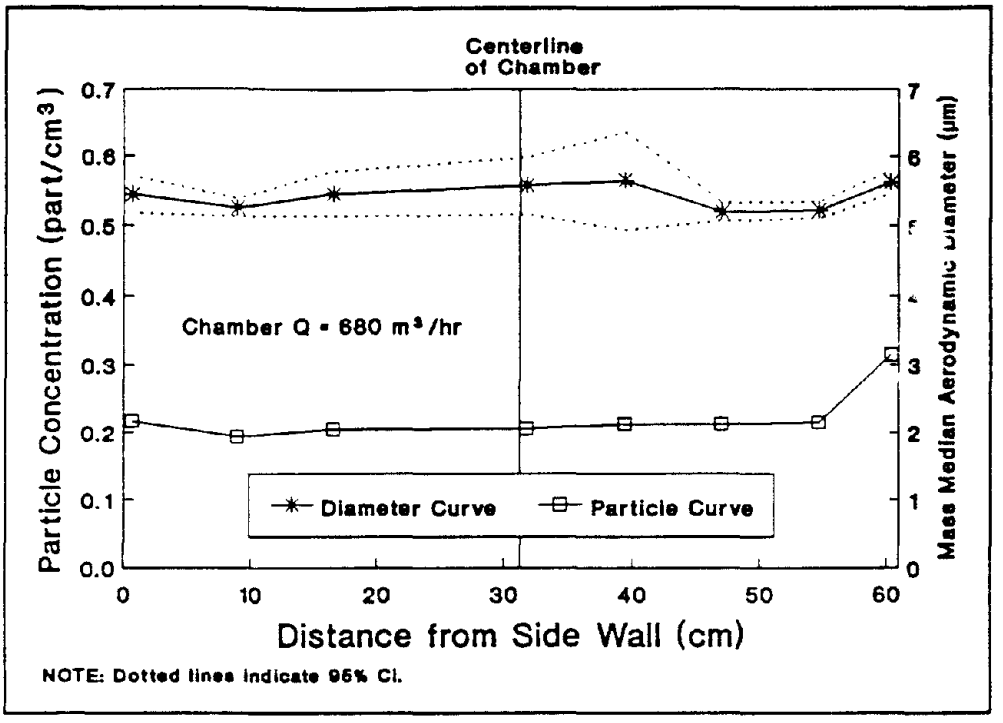

Figure 7. Modified chamber design particle mass concentration and MMAD - horizontal traverse (2.79 m downstream, z-axis, from second baffle and 0.14 m from, x-axis, the chamber side wall).

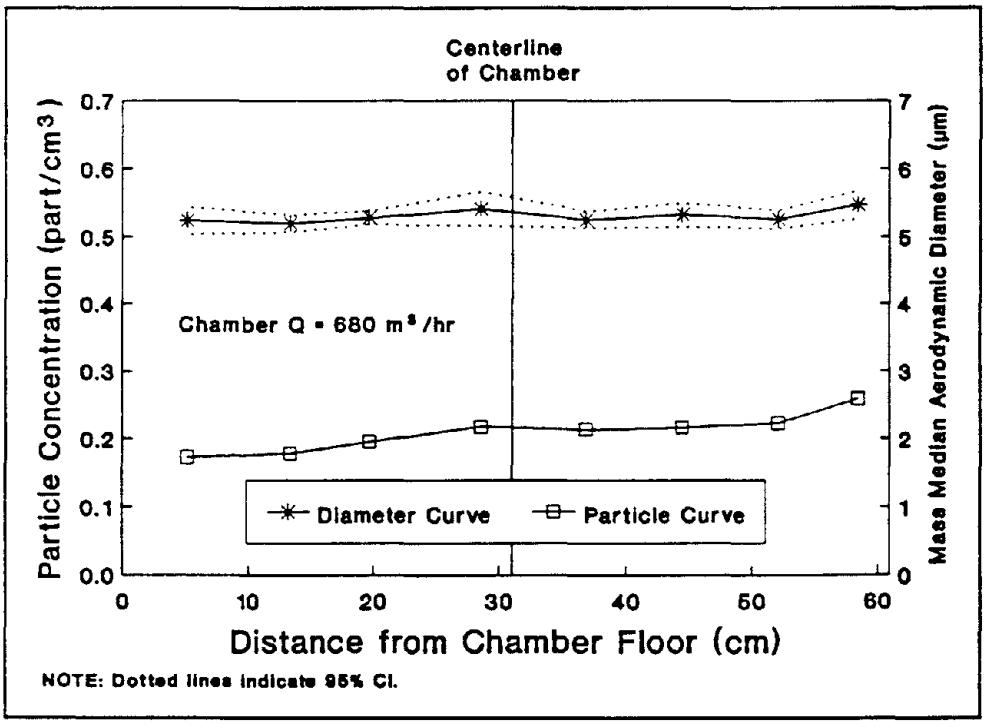

Figure 8. Modified chamber design particle mass concentration and MMAD - vertical traverse (2.79 m downstream, z-axis, from second baffle and 0.30 m above, y-axis, the chamber floor).

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