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EVALUATION OF ALTERNATIVE SAMPLERS FOR BIOAEROSOLS PHASE I: PHYSICAL SAMPLING EFFICIENCY

Performed Under

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ABSTRACT

Biotechnology is one of the major emerging technologies of the 80's and 90's. As commercial aspects of the technology increase and diversify, it is highly desirable to protect workers and the general public from exposure to bioaerosols at the design stage of large-scale production facilities. Assessment of potential worker exposures to the microorganisms used in large-scale biotechnology based production facilities can only be validated by the generation of data collected from "real" industrial processes. However, these data are only as reliable as the sample collection devices. This project compares the sampling efficiency of the Biotest centrifugal sampler, the SAS surface air sampler, the Andersen two-stage sampler, and the Andersen single-stage sampler with the membrane filter sampler. During the first phase (which is reported here), each sampler was challenged with a microbial surrogate aerosol under controlled conditions in a horizontal chamber designed for the study of aerosol samplers. This allowed the evaluation of the instrument sampling efficiencies excluding the effects of microorganism species and survivability. From the conclusions, assessments can be made concerning the feasibility and applicability of these alternative samplers for use in not only the biotechnology industry (specifically, their use in determining process microorganism releases in fermentation type operations) but also in assessing occupational exposures to microorganisms in agricultural environments and indoor air quality investigations. Significant long-term benefits of this project will be the definition of the capabilities and limitations of bioaerosol collection instruments and techniques.

The results indicate that the Andersen two-stage sampler differs from the other samplers except for the reference sampler, the filter cassette, in terms of the level of measurement on the average and by particle size level. Although the Andersen two-stage sampler over sampled the reference sampler, overall, by a bias of 10%, it was not statistically significant. The Andersen single-stage sampler under sampled the reference sampler by 47%, which was a significant difference. For a particle size of 3.25 μm, this sampler under sampled the reference sampler by -10%. Subsequently, at increasing particles sizes of 5 and 10 μm, the sampling efficiency began to fall off with biases of -29% and -89%, respectively. In addition, the observed CV for the Andersen single-stage was the highest of all the samplers at 67% possibly indicating a certain amount of imprecision in this instrument. The Biotest and the SAS sampler both under sampled the reference sampler by -81% and -57%, respectively. Both of these biases were statistically significant. However, it is important to note that for the Biotest sampler the researchers used the total sampling flow rate of the instrument (280 lpm) was used in the computations and not the manufacturers recommended "separation" flow rate of 40 lpm. There was not a statistical difference among particle size ranges for the bias of the Biotest sampler. However, there was a statistical difference among particle size ranges for the bias of the SAS sampler. Specifically, the sampling efficiency of the instrument fell off to -67% and -64% at 3.25 and 10 μm, and respectively, from -34% at a particle size of 5 μm.

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I. INTRODUCTION

Biotechnology is one of the major emerging technologies of the 80's and 90's. As commercial aspects of the technology increase and diversify, it is highly desirable to protect workers and the general public from exposure to bioaerosols at the design stage of large-scale production facilities. Both the Engineering Control Technology Branch (ECTB) - Division of Physical Sciences and Engineering (DPSE) of NIOSH, and the Risk Reduction Engineering Laboratory (Office of Research and Development - ORD) of the Environmental Protection Agency have research interests in the efficacy of the containment of processes which use genetically modified organisms. ECTB has completed an assessment of production scale fermentation processes to determine actual levels of biological emissions. The data from this work has been transmitted from DPSE to EPA as part of a current interagency agreement with EPA. The primary reason for this project, however, involves the development of improved bioaerosol sampling techniques to generate worker exposure data to microbial agents collected in the field. A greater understanding of bioaerosol sampling techniques and sample analysis is needed to develop standardized guidelines for use in the biotechnology industry.

Assessment of potential worker exposures to the microorganisms used in large-scale biotechnology based production facilities can only be validated by the generation of data collected from "real" industrial processes. However, these data are only as reliable as the sample collection devices. The Andersen 6-stage cascade impactor, the Slit-to-agar sampler, and the AGI-30 (all glass impinger) have, in the past, been the primary sampling methods for the enumeration of viable microorganisms. However, these sampling methods have inherent deficiencies that make them only marginally effective for evaluating industrial bioprocesses. Although the Andersen 6-stage and the AGI-30 accomplish the task of collecting "reliable" microbial data, the time involved to collect the samples or to conduct the final microbiological analyses make them "inconvenient" and costly for field work. The Andersen 6-stage is labor intensive and not very portable when collecting samples in an industrial setting. The deficiencies of portability are due, to a large extent, to the requirement of a standard AC current to power the sampling pump. The Andersen 6-stage is also not applicable as a personal sampling device. Sample times for the Andersen range from a few minutes to 30 minutes, depending on the intended purpose of the sample and the air sampling environment. The slit-to-agar sampler also requires an AC power source The AGI-30 is portable (when compare to the Andersen six-stage sampler) but sample times are limited to short intervals. Analysis of impinger samples are also labor intensive. Samplers for viable microbiological aerosols and their basic principles of operation are briefly described in Table 1.[1,2,3]

The accurate sampling of vegetative microbial cells is a precarious function based on their fragile nature. Requisite air streams produced by sampling pumps contain large amounts of oxygen (which may be inimical to certain facultative anaerobic species), and airborne chemicals may react with cells in such a manner as to attenuate viability

Table 1. Samplers for the Collection of Microbiological Aerosols

Sampler Type	Principles of Operation	Sampling Rate (liters per minute)
Slit-to-agar impactor	Impaction onto rotating plate or tape; viable counts in terms of CFU/m^3; provides microbial concentration over time	10-50 continuous
Sieve impactor		
a. Andersen six-stage impactor	Impaction onto agar; six 100x15 mm plates; viable counts in terms of CFU/m^3	28.4
b. Andersen two-stage impactor	Impaction onto agar; two 100x15 mm plates; viable counts in terms of CFU/m^3	28.4
c. Andersen single-stage impactor	Impaction onto agar; single 100x15 mm plate; viable counts in terms of CFU/m^3	28.4
d. SAS single-stage impactor	Impaction onto agar; single RODAC plate; portable; viable counts in terms of CFU/m^3	90 or 180
Biotest Reuter centrifugal impactor	Impaction onto agar; single plastic strip with series of wells; portable; viable counts in terms of CFU/m^3	280
All-glass impinger	Impingement into liquid; viable counts in terms of number of microorganisms (i.e. bacteria) per m^3 or CFU/m^3	12.5
High-volume electrostatic	Electrostatic collection into liquid	up to 1000
Filter cassette	Filtration; viable counts in terms of CFU/m^3; non-viable counts available if analyzed microscopically	1-2
High-volume filtration	Filtration; used for collection of low levels of antigens	140-1400

Note: CFU/m^3 - Colony Forming Units per cubic meter of air.

or cause their destruction. Desiccation of cells can be a serious problem with dry collection methods.[4,5]

There is a multitude of documentation available evaluating the comparability of alternative methods of air sampling for viable microorganisms. Included among these alternatives are the Andersen 2-stage viable sampler, the Andersen single-stage sampler, the membrane filter sampler, the Biotest centrifugal sampler, and the Surface Air System (SAS) sampler. A comparison of the 2-stage and 6-stage impactors by Gillespie et. al. indicated that the former sampler gives lower readings than the latter when aerosol particle concentrations greater than 1 μm in diameter are less than 1000 per cubic meter of air.[6] Jones et. al. compared the Andersen single-stage sampler with the Andersen six-stage sampler under field conditions and determined that the samplers produced similar results.[7] Smid et. al. compared four samplers under field conditions including the Andersen single-stage sampler, the Biotest centrifugal sampler, the slit-to-agar sampler, and the SAS sampler. Smid concluded that the SAS sampler under-estimated the alternatives by approximately 50% and the Biotest sampler, overall, is less accurate than the alternatives.[8] Other case studies compare the remaining samplers to the slit-to-agar sampler.[9,10,11,12,13]

This project compares the sampling efficiency of the Biotest centrifugal sampler, the SAS surface air sampler, the Andersen two-stage sampler, and the Andersen single-stage sampler with the membrane filter sampler. The study is separated into two phases. During the first phase (which is reported here), each sampler was challenged with a microbial surrogate aerosol. This allowed the evaluation of the instrument sampling efficiencies excluding the effects of microorganism species and survivability. The second phase of the study will be conducted under the same experimental conditions except the sampling instruments will be challenged with a viable microorganism. Both phases were conducted under controlled conditions in a horizontal chamber designed for the study of aerosol samplers. The results were to be statistically evaluated to determine the reliability of these samplers. Statistical comparisons were also conducted to compare samplers with each other, in addition to the reference sampler, the 37 millimeter (mm) open-faced filter cassette.

II. SAMPLER DESCRIPTION

A. ANDERSEN SINGLE-STAGE AND TWO-STAGE CASCADE IMPACTORS

The Andersen single-stage and two-stage bioaerosol samplers are multi-orifice, cascade impactors which are used to measure microorganism concentrations and particle size distributions in the ambient environment. The microorganisms can include aerobic bacteria and fungi based on the particular nutrient agar used as the collection media. For the Andersen two-stage sampler (see Figure 1), the air sample is drawn through a series of 200 holes (400 holes for the single-stage model) and is directed towards an agar filled petri dish. Large particles, typically found to be in the non-respirable range (estimated 50% cutoff diameter at 8 micrometers (μm) for spherical particles of unit density), are impacted onto the agar surface of stage 1. Smaller (respirable) particles remain entrained in the air stream and are re-directed around the petri dish through another series of 200 holes of smaller diameters than those found on Stage 1. These smaller particles are then impacted onto the agar surface of Stage 2. Both samplers are designed to operate at a flow rate of 28.3 liters per minute (lpm). The Andersen six-stage is constructed so that the collected bioaerosols can be directly related to human respiratory system deposition irrespective of size, shape, or density.[14] The particle size ranges for the Andersen six-stage are listed in the following table along with the corresponding deposition regions of the human respiratory system. This project evaluated the last (6th) stage of the Andersen six-stage sampler. The Andersen six-stage was not included for evaluation.

Table 2. Andersen Six-Stage Sampler Particle Size Ranges[14]

STAGE	SIZE (μm)	RESPIRATORY PENETRATION
1	7+	non-respirable
2	4.7 - 7	pharynx
3	3.3 - 4.7	trachea and primary brachii
4	2.1 - 3.3	secondary brachii
5	1.1 - 2.1	terminal brachii
6	0.65 - 1.1	alveoli

The premise behind the Andersen microbial aerosol collection method is based, in part, on inertial impaction theory work conducted by Ranz and Wong in the early 1950's.[15]

The collection of a particle by an obstacle is stated to be a function of the inertial impaction parameter:

$$K = \frac{C_c \rho U D_p^2}{18 \mu D_c}$$

where U is the relative velocity of air in the jet, ρ is the particle density, D_p is the particle diameter, μ is the gas viscosity, D_c is the diameter of the round jet, and C_c is the Cunningham slip correction factor. Andersen impactors have an inertial impaction parameter K of 0.14 for a 50% effective cutoff diameter. The collection efficiency for the Andersens is stated to be slightly greater than that predicted by inertial impaction theory for particle diameters greater than 1 to 2 μm. The specific design of the samplers minimizes the tendency of overlapping particle sizes between stages that is a common occurrence among cascade impactors.

B. SURFACE AIR SYSTEM

The Surface Air System (SAS) bioaerosol sampler is similar to the Andersen impactors. In addition, the SAS unit is portable and SAS can sample for bacteria and fungi based on the particular nutrient media used. Ambient air is drawn through a series of holes and directed towards an agar filled Replicate Organism Detection and Counting (RODAC) plate (refer to Figure 2). Based on inertial impaction theory, larger particles are impacted onto the agar surface. The remaining particles are exhausted out the back of the sampler. The SAS aspirates air at the rate of 90 lpm for the compact model (used for this study) and 180 lpm for all other sampler models. Lach [1985] evaluated the volumetric air flow into two larger model SAS samplers (quoted flow rates of 180 lpm) by attaching a 100 mm diameter tube to the sampler inlet and extracting measurements with a vane anemometer. Lach measured values 10-20% greater than the quoted flow rate of 180 lpm but attributed the differences to the methods of measurement.

C. BIOTEST REUTER CENTRIFUGAL

The Biotest Reuter Centrifugal bioaerosol sampler is similar to the Andersen and SAS samplers in that it uses inertial impaction to collect viable microorganisms onto a nutrient agar surface. Like the SAS sampler, the Biotest is a portable unit weighing 2.5 pounds. The Biotest can sample for bacteria and fungi, but due to the small size of each well in the agar strip and the tendency for fungi to rapidly overgrow the culturing area, it is not recommended for the enumeration of fungi. Ambient air is sampled concentrically and conically by an impeller drum (see Figure 3). The incoming air is set into rotation and centrifugally forced past an agar filled plastic strip. Larger particles are impacted onto the agar surface. The air that leaves the sampler is set into a spiral motion outside of the envelope of the incoming air.

The Biotest unit has special volume characteristics that require the differentiation between total volume sampled (sample volume) and the volume that is used for separating bioaerosols (separation volume).[16] The separation volume is used for calculating the number of microorganisms (Colony Forming Units - CFU) per volume of air. According to the manufacturer, the separation volume can be determined by mathematical evaluation given the measured velocity profiles of the incoming air over a specified radius. The Sample Volume is 280 lpm at an impeller rotational rate of 4096 revolutions per minute. The separation volume is calculated mathematically based on the height of the instrument head (l_{min}) for the separation of all particles in the sample volume. This is based on the solution to a differential equation which models the spiral path of the sampled particles influenced by air flow velocity, air flow direction, and the resultant centrifugal force. The l_{min} is determined by the following equation:

$$l_{min} = \frac{1}{2\pi b a^2 \omega^2} \left[\frac{18\eta b}{D_p^2 \rho} V - \frac{V^2}{2\pi a h} \right]$$

where ω is the angular velocity, η is the viscosity of the air, D_p is the diameter of the particle, ρ is the density of the particle, and V is the Sample Volume. A particle of a diameter of 4 μm produces a height of 14 cm. However, the manufacturer claims that only 2 cm of the instrument are available for particle separation, therefore the Separation Volume is 40 lpm.

Clark et. al. [1981] investigated the air flow characteristics of the Biotest sampler by means of a thermoanemometer. Additionally, a direct estimate of the volumetric flow rate was made by placing a 6 cm diameter tube above (and coaxial with) the impeller and using a vane anemometer at the far end of the tube to determine inflow. They determined the effective sampling volume (the manufacturer's separation volume) to average approximately 100 lpm. A similar study conducted Macher and First was based on a design of a non-intrusive inlet adapter that would separate the inflow and outflow airstreams of the Biotest sampler.[17] With the adapter in place, the airflow through the sampler was measured at 210 ± 27 lpm. This measured airflow is dramatically different from the manufacturers separation volume of 40 lpm. Macher and First state that:

> "From the standpoint of the manufacturer, it is preferable to state that the instrument removes 100% of 4 μm particles from a 40 liter/min fraction of the total sampled air than to state that the instrument is 14% (40/280) efficient for collecting 4 μm particles, based on the total airflow induced into the sampling chamber."

D. OPEN-FACED CASSETTES

The open-faced cassette is an air sampling device used for the collection of various hazardous chemicals in the occupational environment. Techniques for the application of the membrane filter cassette to the sampling of microbial aerosols have been described in the literature.[18,19,20] Differences exist in the sampling and analytical techniques that specialize the sampling method for the collection of microorganisms in the ambient air. Caution dictates that the sampling times for this instrument remain brief, due to the high probability of desiccation of microbial cells on the filter assembly. This condition may result in the gross under reporting of viable counts. For desiccation sensitive microorganisms, the Open-Faced Cassette may prove to be more of a qualitative as opposed to a quantitative indicator of microbial levels in the ambient air. Air samples are collected on a 37 millimeter, 0.8 μm pore size, polyvinyl chloride filter enclosed in a polycarbonate cassette assembly (see Figure 4). Air is drawn through the filter where particles are collected. Small air flows help to minimize the effects of cell desiccation. After sampling, the filter is removed from the cassette and inverted onto an agar surface in a petri dish or the filter is rinsed with a sterile solution. Counts are based on the colonies observed on the filter or the number of cells (and/or spores) in solution. Other methods of analyses include acridine orange staining with epifluorescence microscopy and electron microscopy.[21,22]

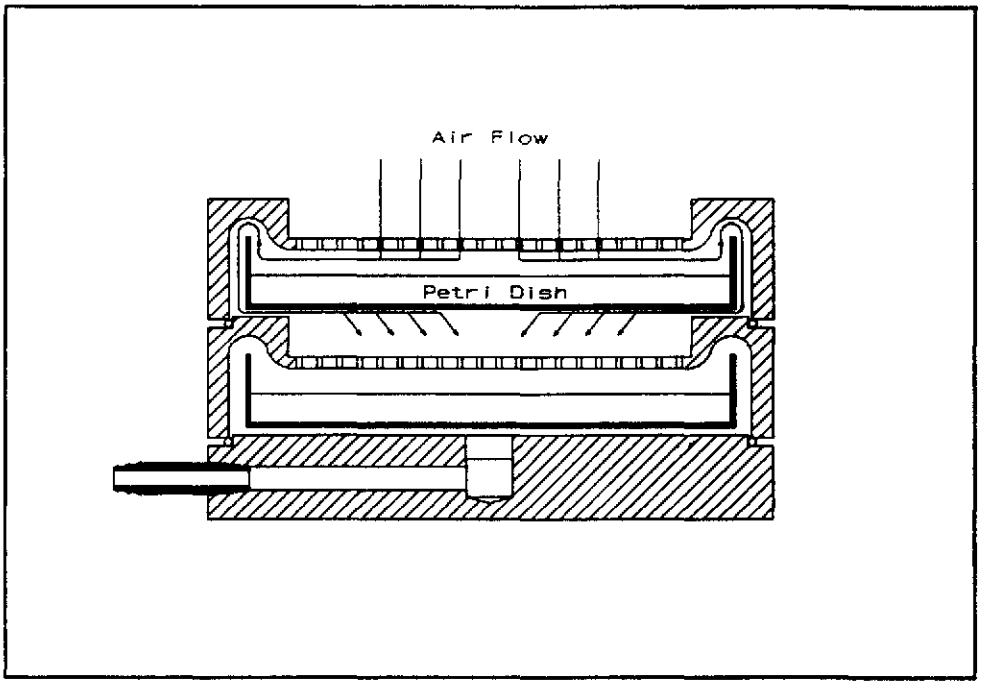


Figure 1. Andersen Two-stage Cascade Impactor

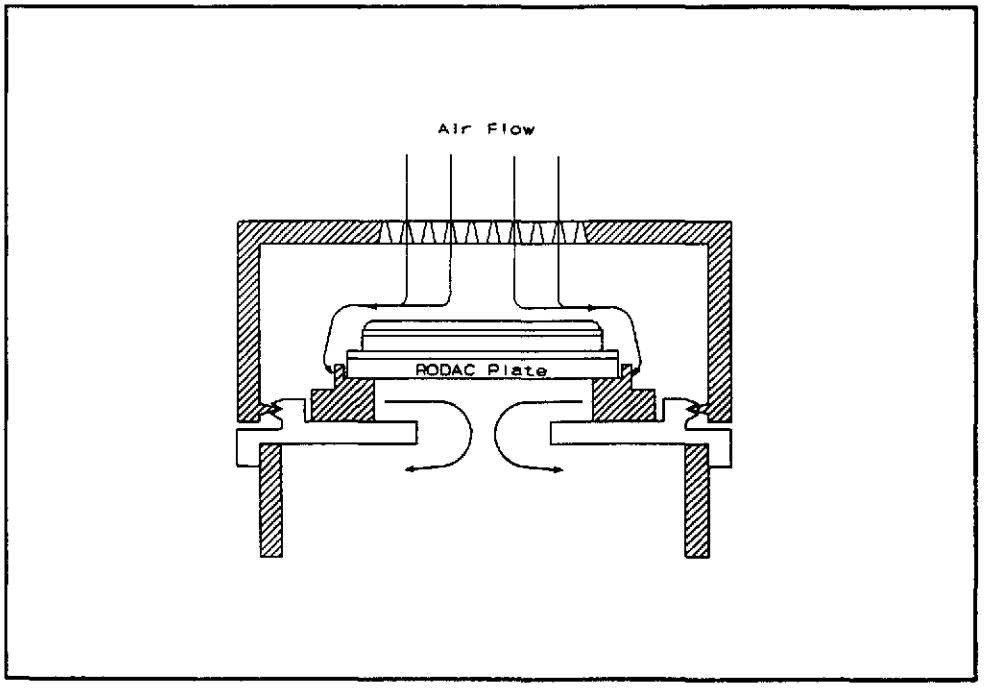


Figure 2. SAS Surface Air Sampler

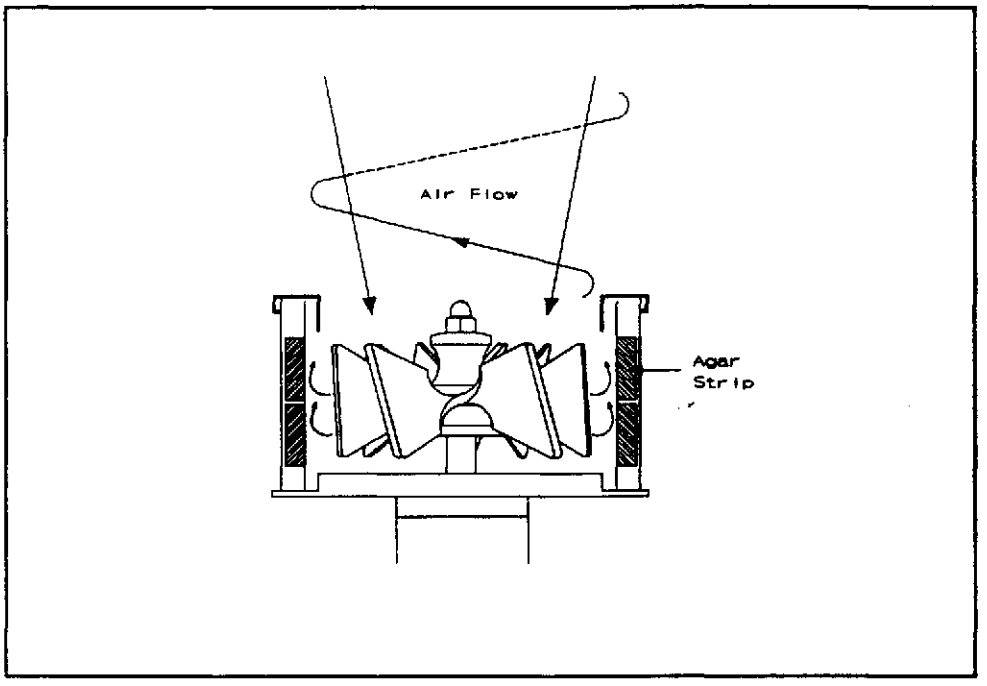


Figure 3. Biotest Reuter Centrifugal Sampler

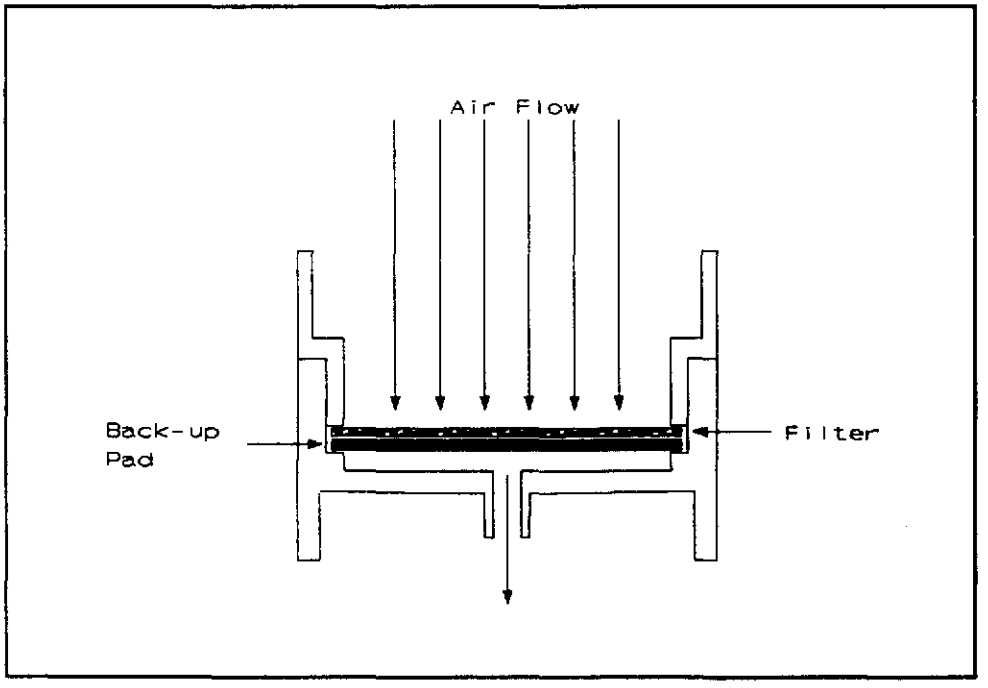


Figure 4. 37 mm Filter Cassette

III. OBJECTIVES AND HYPOTHESIS

The primary objective of this phase of the project was to determine the variability of particle collection efficiencies of bioaerosol sampling methods. The resultant data was used to determine sampling efficiencies for the individual samplers included in the study. From the conclusions, assessments can be made concerning the feasibility and applicability of these alternative samplers for use in the biotechnology industry (specifically, their use in determining process microorganism releases in fermentation type operations), occupational exposures to microorganisms in agricultural environments, and indoor air quality investigations. Significant long-term benefits of this project will be the definition of the capabilities and limitations of bioaerosol collection instruments and techniques.

To accomplish the primary objective, determinants of the ability of each sampler to detect the general ambient microorganism concentration under specific conditions were identified and evaluated. Some of the identified determinants were not readily quantifiable, if at all. For example, temperature and humidity were measurable but not controllable variables. Determinants include:

Environmental Air (Direction and Speed of Air Movement) - relative vector of airstream motion of the ambient sampling atmosphere

Particle Size Distribution - frequency distribution of the sizes of the particles in the ambient air stream

Temperature - degrees centigrade of sampled air

Humidity - relative water content of air as determined by wet and dry bulb readings (psychrometer)

Surrounding Activity - natural occurrences (e.g., meteorological, radiant energy), human events, evolution of process by-products, etc., that affect the content, quality or other measurable parameters of the sampled air, and of which the sampling personnel have little or no control

Microbial Viability - capability of vegetative proliferation and the capacity to elicit competent biochemical responses of the given species

Sampler Sterility Between Samples - elimination of vegetative or spore-forming capacity of microorganisms on critical, i.e., sampling contact surfaces, by autoclaving or chemical means

Operator Sampling Technique - performance of sample collection, handling, storage, and analyses, according to standardized or accepted practices

Consideration of all the determinants was instrumental in evaluating a working hypothesis of this research project. However, a systematic evaluation of each determinant, excluding the effects of the others, can offer more insight into the sampling efficiencies of each instrument. For the first phase of this research project, a working hypothesis that summarizes the primary objective can be stated:

> "Each bioaerosol sampler alternative is capable of reproducing the results obtainable with a reference sampler over a range of particle sizes under the same experimental conditions."

This hypothesis was tested for physical sampling efficiency by performing independent comparisons of four samplers, challenged with a microbial surrogate aerosol, to a reference sampler, the filter cassette. The 37 mm filter cassette, under "calm wind" conditions, has been shown to exhibit a sampling error of less than 20% for particles less than 15 μm in diameter.[23] The samplers include the Andersen single-stage, the Andersen two-stage, the Biotest, and the SAS. Each sampler was studied to determine the presence of systematic differences or bias, if any, in the measured concentrations of aerosol sampled relative to the filter cassette. The null hypotheses to be tested are that the differences between the control and alternative sampler means is zero over a range of particle sizes. The data for all of the samplers were then evaluated to determine if there are systematic differences among the four samplers in measured aerosol concentrations. The null hypothesis tested is that the differences among the four bioaerosol samplers is zero over a range of particle sizes.

IV. CONDUCT OF EVALUATIVE EXPERIMENTS

A. EXPERIMENTAL DESIGN

Individual bioaerosol sampler efficiency assessments were made in a horizontal aerosol chamber designed to provide a constant air velocity profile across the chamber face. Each sampler was placed in the aerosol chamber with the filter cassette and the chamber parameters were then set to the desired experimental conditions. Three experimental conditions were studied by varying particle size at three levels of 3.25, 5, and 10 μm, designated through out the remainder of the document as "3", "5", and "10", respectively.

Experimental study blocks were designated over half day intervals, morning or afternoon, and two samplers were studied in any given morning or afternoon. However, each member of the pair was studied independently of the other except that the same particle size level was used for each half day. Two samplers were selected for study in any given day. All six possible pairs were studied in a randomized order. Three pairs were placed in another randomly selected order for an additional cycle of study. This was repeated for three complete cycles. However, on the first cycle, the pair was studied only for either a morning or an afternoon session, but not both. For the first cycle of 6 half days, particle size was set at 5. For the last two cycles, the morning and afternoon were randomly assigned to particle size sequence of 3 then 10, or 10 then 3. In each sampling run a bioaerosol sampler was placed in the chamber along with the filter cassette and an aerosol was generated at a specific particle size (the measured concentrations were recorded). This procedure was repeated twice more for the same sampler, which produced a total of three paired observations for the sampler and the filter cassette. These two steps were repeated for the other sampler to produce three observations for both samplers each paired with three observations for the filter cassette.

B. EXPERIMENTAL EQUIPMENT

The experiment involved the generation and collection of a known quantity of a microbial surrogate aerosol (oleic acid tagged with a fluorescent dye - uranine) inside of a controlled environment, an aerosol chamber. In this phase of the project, the use of microorganisms was excluded to remove the variability due to microbial viability. The configuration of the aerosol chamber allows the control of air velocity, air direction, aerosol particle size and concentration, and any influence from surrounding activity. Temperature and humidity were uncontrollable variables determined by the quality of the laboratory air (which for this experiment remained fairly consistent). All inlet and outlet air was purified with a High Efficiency Particulate Air (HEPA) filter. This inhibits the contamination of the inside of the chamber with ubiquitous atmospheric particles and also minimizes the release of the study aerosol into the general environment.

1. Aerosol Chamber

A diagram of the aerosol chamber is shown in Figure 5. The chamber is composed of a stainless steel duct of approximately 12 feet in length and having a square cross section of 2 feet. It was designed with the flexibility to allow modifications that may be required by the second phase of this study (i.e., the use of microbial aerosols and the incorporation of a sterilization system into the chamber). The following specific features have been incorporated into the design:

* Stainless steel construction to facilitate cleaning and reduce corrosion
* Baffle plate at the inlet to cause aerosol-air mixing
* Flow straightener to produce laminar streams
* High efficiency particulate air (HEPA) filters at inlet and outlet
* Variable rate fan
* Observation port

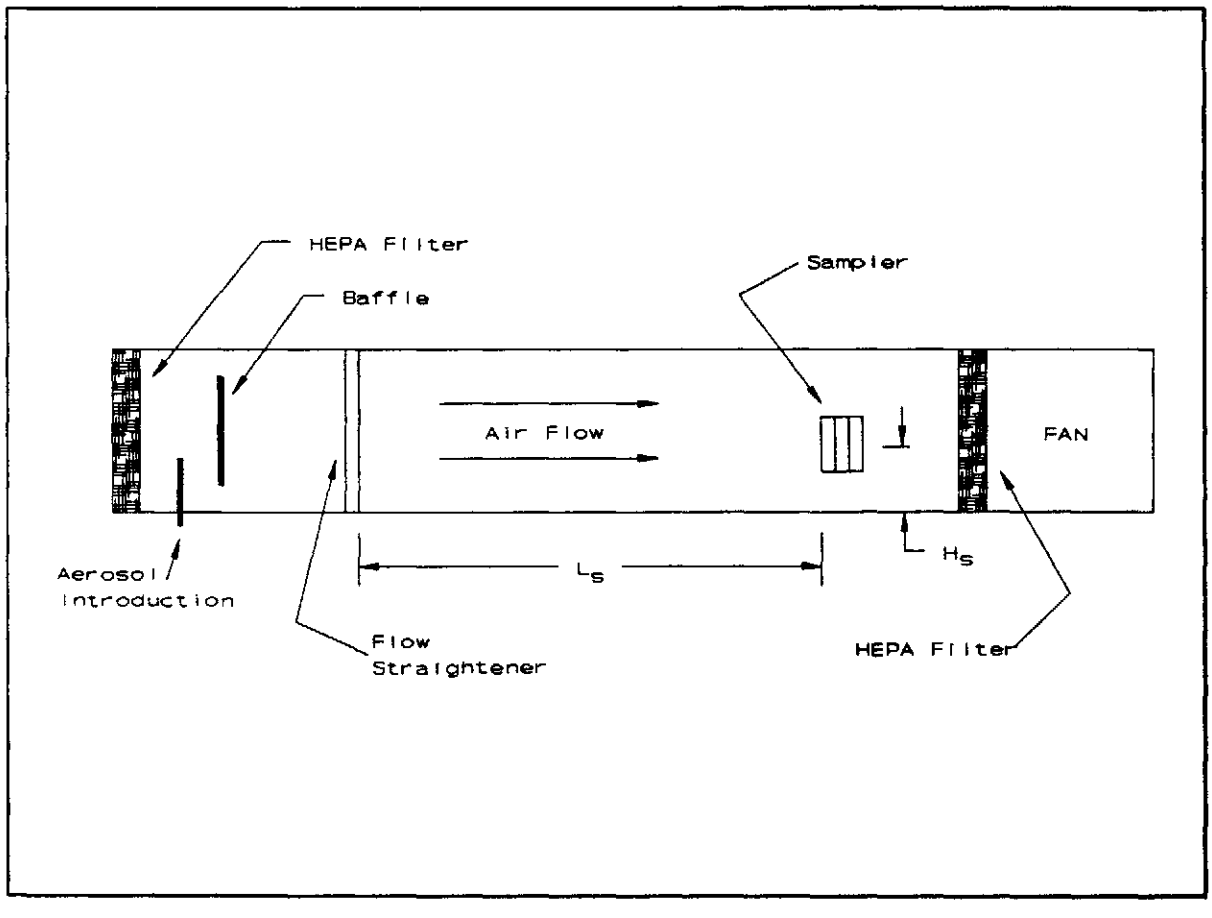


Figure 5. Aerosol Chamber

The aerosol chamber was designed to give a velocity at the sampler face of approximately 50 feet per minute (fpm). This low velocity falls within the still-air sampling criteria (reminiscent of real-world conditions) as defined by the following equation:[24]

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

where U_0 is the maximum air velocity for which the still-air sampling criteria can be used, Q is the sample flow rate in cm^3/sec, and τ is the relaxation time for a given particle diameter. For a 10 μm particle diameter, the maximum velocity limit is approximately 240 fpm.

A velocity profile in the chamber was obtained by completing a traverse across the horizontal and vertical centerlines of the chamber face. Profiles were obtained at 3 and 8 feet downstream of the baffle plate and are shown as velocities (fpm) along traverse points in Table 3. Note, the traverse extends from the wall of the chamber to the centerline.

Table 3. Velocity Profiles within Aerosol Chamber

	Relative Position Along Traverse											
Traverse Angle	1"	2"	3"	4"	5"	6"	7"	8"	9"	10"	11"	12"
Velocity (fpm) 3 feet Downstream from Baffle Plate												
Horizontal	32	40	32	29	24	20	18	28	30	27	20	24
Vertical	68	50	45	36	35	32	25	30	32	25	25	25
Velocity (fpm) 8 Feet Downstream from Baffle Plate												
Horizontal	55	45	50	45	55	45	50	50	50	45	45	40
Vertical	70	65	50	45	50	50	50	45	38	38	40	40

Unlike a Marple chamber, the length of the chamber used in this study has a horizontal axis.[25] 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.[26] Elutriation, which is an inevitable

consequence of the decay of an aerosol particle trajectory along the critical path of a horizontal duct, was not a problem for the specific particle sizes used in this study. The height that a particle will settle over the chamber length from the baffle plate to the sampler location (L_s) is given by the equation:[24]

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

where V_{ts} is the terminal settling velocity at a given particle size and $V_{chamber}$ is the air velocity in the chamber (approximately 50 fpm). Based on this equation, the largest particle size (10 μm) will settle 1.1 inches from the baffle plate to the sampler.

2. Aerosol Generation

A Berglund-Liu vibrating orifice monodisperse aerosol generator was used to produce aerosol particles of fluorescently tagged (with uranine) oleic acid.[27,28] The aerosol generator operates through the combined contributions of four parts: the liquid feed system, the droplet generator, the droplet dispersion system, and the aerosol flow system. Figure 6 shows a schematic of the generation system. The liquid feed system forces a liquid (for this study the liquid consists of a mixture of uranine, 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 size of the particles studied were 3.25, 5, and 10 μm in diameter. One size category was used per sample run, in order to expose the samplers to a monodisperse aerosol. Due to the importance of keeping the orifice disc clear of particulate matter, all reagents were of spectroscopic grade and underwent filtration. The initial stock uranine/alcohol solution was filtered twice through a 0.45 μm filter. The combined mixture of uranine, alcohol, and oleic acid was filtered four times

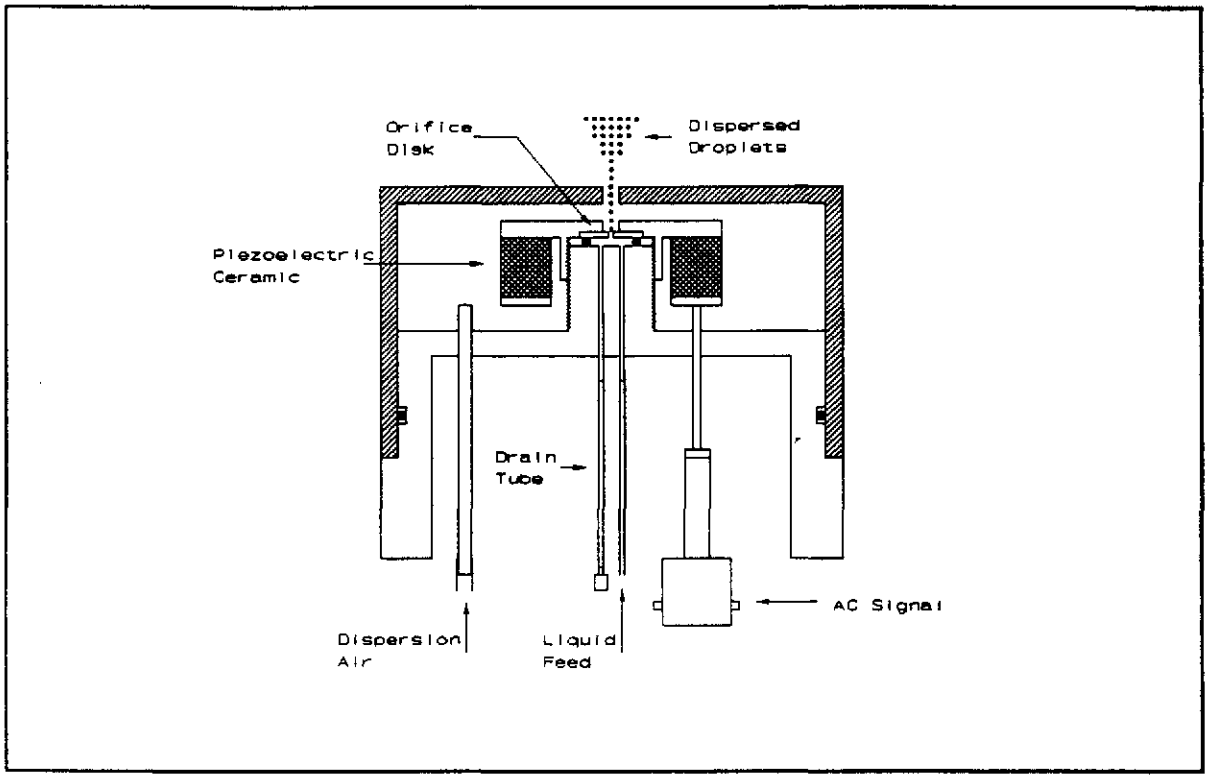


Figure 6. Schematic of Berglund-Liu Vibrating Orifice Monodisperse Aerosol Generator

through a 0.45 μm filter prior to injection into the aerosol generator. A series of static eliminators were placed around the base of the dilution column (approximately 2 inches above the point of aerosolization) to neutralize the charge of the aerosol particles before entering the chamber.

C. VERIFICATION OF PARTICLE CONCENTRATION AND AERODYNAMIC DIAMETER

Actual aerosol concentration at each sampler location was validated by the 37 mm filter cassette. These samples were analyzed by the same fluorometric method that was used for the bioaerosol samplers. The original concentration of uranine in each aerosol particle in conjunction with the results from the filter cassette were converted to the number of particles/cubic meter of air -- this is similar to the results obtained by the bioaerosol samplers when sampling for viable microorganisms (CFU/m^3). In addition to the calculations and accompanying instrument adjustments that were made to form monodisperse aerosol particles of a given aerodynamic diameter, a periodic check was performed for each sampling run with an Aerodynamic Particle Sizer (APS 33B, TSI, Inc. Minneapolis, MN) which provided real-time sensing.

D. SAMPLING

The evaluation of each sampler was conducted individually in the aerosol chamber for time periods ranging from 5 to 15 minutes (depending on the sampler). Sampling times were selected so that each bioaerosol sampler collected approximately the same volume of air. Concurrent sampling was conducted with a 37 mm filter cassette and the APS to verify particle concentration and aerodynamic diameter. An epoxy resin was substituted for the nutrient medium that would be used in a real-world situation. The phenomenon of particle bounce was assumed to be negligible because of the liquid nature of the aerosol particles. Quantification of the deposition of the surrogate particulates were facilitated by fluorometric methods.

Each sampler was oriented in the chamber so that the midline of the sampler intake nozzle is facing the aerosol generator output source at a pre-determined reference point. Note, this particular sampler orientation resulted in isoaxial sampling. Isokinetic sampling was not possible due to the samplers, filter cassette, and APS all operating at different flow rates. In addition, the criteria of isokinetic sampling are not applicable because of the still air sampling assumption. The aerosol generator was activated for 1 minute, in order to achieve a stable aerosol concentration. Subsequently, the sampler was activated and operated for the given sampling period.

1. Andersen Single-stage and Two-stage Cascade Impactors

The Andersen single-stage and two-stage cascade impactors were individually connected to a continuous duty vacuum pump that was operated at a flow rate of 28.3 lpm for a period of 15 min. The Andersen single-stage was used with one 100 millimeter (mm) x 15 mm plastic pre-sterilized disposable dish, each filled with 60 ml of epoxy resin. This approximates the equivalent height of the sampling surface if a glass petri dish had been filled with 20 ml of agar. The two-stage impactor requires the use of two of the aforementioned dishes filled with 20 ml of epoxy resin. Due to the horizontal orientation (parallel to the plane of the air flow) of the sampler, an adhesive material was used on the bottom of the sample plates (where they contact the sampling instrument) to keep them from moving in the sampler. To ensure the cleanliness of the sampling instruments, they were thoroughly rinsed with de-ionized water and dried with filtered air prior to each sampling block. A sampling block was defined as a series of 3 sampling runs made for each sampler at a specific particle size. Subsequent to sampling, the epoxy resin surface was rinsed with 10 ml of de-ionized water to recover any collected fluorescently tagged oleic acid aerosol particles for fluorometric analysis.

2. Surface Air Sampler (SAS)

The SAS sampler was operated at a flow rate of 90 lpm for a period of 5 min (6 aspirating units on the instrument selector). The instrument requires the use of one 65 mm x 15 mm RODAC plate filled with nutrient agar. An epoxy resin was substituted

for the nutrient agar in the RODAC plate. To ensure the cleanliness of the sampling instrument, the cover was thoroughly rinsed with de-ionized water and dried with filtered air prior to each sampling block. Subsequent to sampling, the epoxy resin surface was rinsed with 10 ml of de-ionized water to recover any collected fluorescently tagged oleic acid aerosol particles for fluorometric analysis.

3. Biotest Reuter Centrifugal

The Biotest operates at a total sample volume of 280 lpm. The Biotest requires the use of one manufacturer specific plastic strip that is filled with nutrient agar. For the purposes of this experiment, the strips were emptied and covered with plastic tape. The sampler exterior and the impeller blade were washed with de-ionized water and dried with filtered air before each sampling block. Subsequent to sampling, the strip surface was rinsed with 10 ml of de-ionized water to recover any collected fluorescently tagged oleic acid aerosol particles for fluorometric analysis.

4. Open-Faced Cassette

The open-faced cassette was operated at a flow rate of 20 lpm of air. One pre-assembled unit (with a 0.45 μm Teflon filter) was used for each sampling run. The cassette face was removed prior to sampling and placed in a clean location to prevent interior contamination. After sampling, the cassette face was re-attached to the cassette base unit. Subsequent to sampling, the filter was immersed in 10 ml of de-ionized water to recover any collected fluorescently tagged oleic acid aerosol for fluoroscopic analysis.

E. SAMPLE ANALYSIS

Fluorometric analysis is very sensitive (which was a necessity for the dilute solutions encountered in this experiment).[29] The method also lends itself to simplicity and specificity. Since there is a linear relationship between concentration and fluorescence, it is possible to construct a calibration curve based on a standard. A 10 ml solution of 2% oleic acid in 10 μg/ml uranine and alcohol mixture was heated until the alcohol had evaporated completely. The remaining solution was then combined with de-ionized water at five dilutions of 0.01, 0.005, 0.001, 0.0005, and 0.0001 μg/ml. A calibration curve was obtained before each days sampling runs. An example of a typical calibration curve is shown in Figure 7.

During the process of aerosolization from the vibrating orifice aerosol generator, the alcohol volatilizes leaving fluorescently tagged oleic acid particles. These particles can then be quantified in solution based on the resultant fluorescence produced. The method is Ph sensitive (optimal pH is approximately 10). A Perkin-Elmer fluorescence spectrophotometer (Model 650-40) was used to quantify the amount of aerosol that had been collected by a given sampler. This instrument is microprocessor controlled and designed to measure fluorescence excitation and emission spectra. Data output

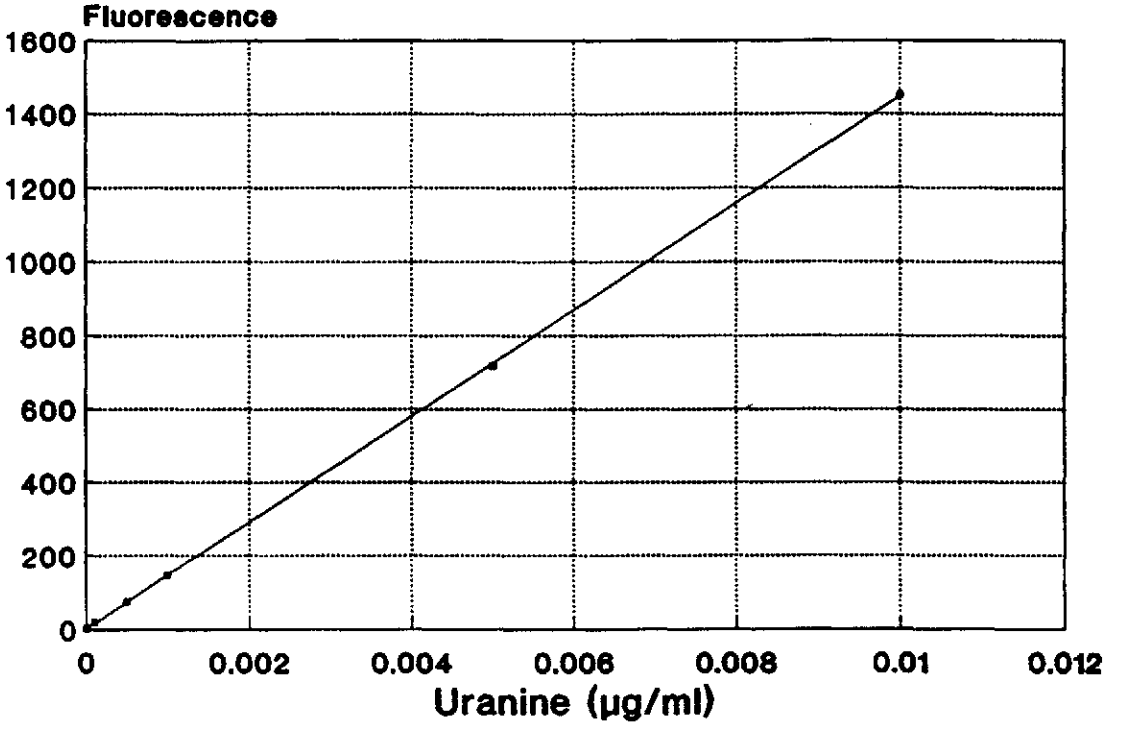


Figure 7. Typical Calibration Curve.

is available through both digital and strip recorder modes. The digital results were converted to units of $\mu g/m^3$ of air based on the calibration curve.

The relationship between fluorescence intensity and concentration has well been described:[30,31]

$$(S_f)_\lambda = f(\theta)\ g(\lambda)\ I_0\ \phi_f\ abc\left[1 - \frac{abc}{2!} + \frac{\overline{abc}^2}{3!} - \cdots + \frac{\overline{abc}^n}{(n+1)!}\right]$$

where $(S_f)_\lambda$ is the sample fluorescence intensity at a given wavelength, $f(\theta)$ is the geometry depending on the effective solid angle, $g(\lambda)$ is the response characteristic of the detector (varies with wavelength), I_0 is the intensity of the exciting radiation, ϕ_f is the quantum efficiency of the molecule, a is the molar absorptivity for the sample at the exciting wavelength, b is the sample path length along the axis of irradiation, and c, is the concentration of the fluorescing material in moles per liter.

In situations, as were encountered in this study, where the concentration of fluorescing material is very small ($abc < 0.05$) the equation reduces to:

$$(S_f)_\lambda = f(\theta)\ g(\lambda)\ I_0\ \phi_f\ abc$$

Each sampling surface was rinsed with 10 ml of de-ionized water. In the case of the filter cassette, the filter was immersed in de-ionized water, shaken for 5 min, and then placed in an ultrasonic bath for 1 hour. A 2 ml aliquot was taken from the resultant rinse and poured into a 3 ml cuvette with 3 drops of a buffer solution to bring the pH to approximately 10. The buffer was prepared by dissolving 29 grams of sodium bicarbonate ($NaHCO_3$) and 12.4 grams of sodium hydroxide (NaOH) in 1000 cm^3 of distilled water.[15] All sample aliquots were analyzed twice.. Blanks were obtained at configuration specific series of three sampling runs and subtracted from each individual series result.

F. DATA ANALYSIS

To facilitate analysis, the concentration measurement for the filter cassette was subtracted from the paired measurement for each sampler to calculate the response variable DIFF. Complete agreement in measured levels between a sampler and the filter cassette would imply that all differences are from a population with a mean of zero (the bioaerosol sampler and the filter cassette obtain the same concentration of the measured air or DIFF=0). Agreement, except for a stable or consistent bias, would imply the same result but the mean of the response variable would be non-zero. Systematic differences in measurements (bias due to a particle size effect) would imply at least two populations and perhaps three populations with different means (the three populations being the particle size categories). In the latter case, the overall bias would be less meaningful but could be zero if the average of the means of the populations (particle size categories) were zero.

In all cases, the three replicate observations for a sampler within a specific experimental configuration (i.e., the filter cassette, or the differences in paired observations, for a given one-half day - the block of study) were averaged and all analyses were performed on these averages. Preliminary analysis did confirm the suspected intra-block correlation among the three replicates in the case of each of the four samplers and for all in a combined analysis.

Because of the design, time of day was partially confounded with the particle size (since the effect of particle size was of little importance except in terms of ruggedness of the behavior of the samplers relative to the reference sampler). Preliminary analysis indicated that the effect of time of day (i.e., morning or afternoon) did not approach statistical significance in those cases for which it was testable (the effect was entered after that for particle size). Therefore, time of day was ignored for all analyses and only particle size was included as a factor.

All analyses assume that the differences among the cycles for observing the six possible pairs of samplers are so small as to be practically negligible.

1. Sampler Comparisons to the Reference Sampler

For each sampler, a one-way Analysis of Variance (ANOVA) was used to determine agreement between the sampler and the reference sampler using the response variable DIFF. First, the null hypothesis of no bias is tested by reference to the F-ratio for all effects in the model, i.e. the intercept and the effect of particle size. Lack of significance at the 0.05 level indicates that the bias, if any, is too small to be detected by this study. No further statistical testing is done in that case. A significant F-ratio indicates the presence of some bias.

If the first test indicates the presence of a bias, it could be either a consistent bias of the same magnitude for all particle sizes or that the level of bias varies among the three particle size levels. A significant particle size effect indicates the latter, while lack of significance indicates that the variation in bias, if any, is too small to be detected by this study. If particle size is not significant, it is meaningful to test for a consistent bias by testing the null hypothesis that the grand mean is zero. Significance indicates the presence of a consistent bias whereas lack of significance indicates that the sampler mean level of bias is too small to be detected. The second and third statistical tests are the standard tests for ANOVA. These tests may be non-significant even though the first one is significant, which indicates the presence of some bias that may or may not vary by particle size but cannot be determined.

2. Comparisons Among Samplers

This analysis examines the existence, if any, of bias of the four samplers relative to each other as indicated by differences in measurements. The question is whether such bias is detectable at all and whether it is a constant amount for all particle sizes or whether it varies among particle sizes.

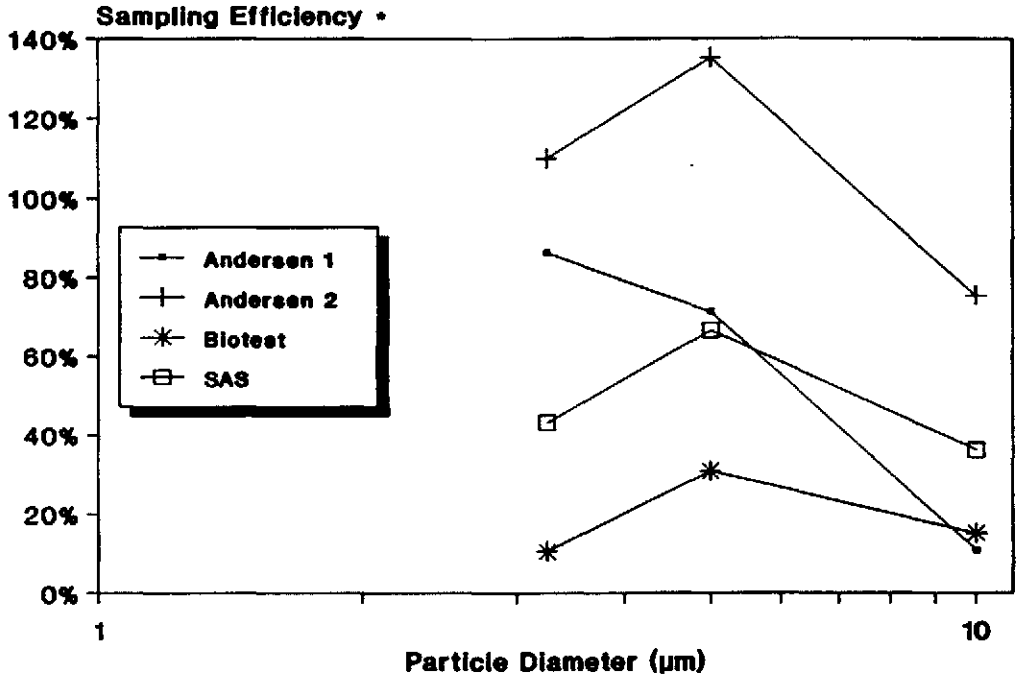
The analysis used ANOVA with the factors of particle size, samplers, and their interaction. The response variable used was the natural logarithm of the measured concentration, to achieve variance stabilization. Although the results were not the same when the measurements were analyzed on the original scale, there was substantial agreement. The analysis assumes that all sources of variation for measurements for two different samplers on two different one-half days were present to the same magnitude for measurements for the same half day. The effect of particle size is not of interest except in terms of its interaction with the samplers indicating a variation in relative bias among samplers among particle size levels.

The presence of any bias is tested by an F-ratio test for the effects of the samplers and the samplers by particle size interaction. Significance, at the 0.05 level, indicates the presence of some relative bias. Lack of significance indicates that the relative

bias, if any, among the samplers is too small to be detected in this study. In the latter case, no further testing is done. If some bias is detected, the presence of variation of relative biases among the particle size levels is tested by the F-ratio test for the samplers by the particle size interaction. If this is significant, variations in bias are present, otherwise such variation, if any, are too small to be detected. If no variation in relative bias among particle size levels is detected, then the sampler effect will be tested by an F-ratio test to determine the existence of bias constant for all particle size levels. The second and third tests may be non-significant even though the first is significant, which indicates the presence of some bias that may or may not vary among particle size but cannot be determined.

V. RESULTS

The individual results of the experimental runs are presented in Appendix A and graphically summarized in Figure 8 as the ratio of the bioaerosol sampler to the filter cassette.



• Ratio of sampler to filter cassette.

Figure 8. Sampling Efficiency of Bioaerosol Samplers

A. SAMPLER COMPARISONS TO THE REFERENCE SAMPLER

A tabular summary of the results is shown in Table 4 for each sampler compared with the reference sampler. The entries in the table include confidence intervals for the overall mean bias and the bias by particle size (bias in either case indicated in the variable DIFF). A negative value for the mean bias (overall or by particle size) indicates an underestimation of the particles in the aerosol chamber as compared to the reference sampler (filter cassette). All confidence intervals are Scheffe's simultaneous intervals for all effects in the model.

Table 4. Summary Results for Bioaerosol and Reference Samplers

Particle Size (μm)	Reference Sampler Mean (p/m³)	Bioaerosol Sampler Mean (p/m³)	DIFF Mean (p/m³)	DIFF Std Error	Confidence Intervals	
					Lower	Upper
Andersen Single-stage Sampler						
Overall	40439	21437	-19001	4199	-34865	-3128
3.25	39105	33774	-5331	7273	-32808	22146
5	35810	25510	-10300	7273	-37777	17177
10	46401	5028	-41373	7273	-68850	-13896
Andersen Two-stage Sampler						
Overall	43628	48016	4388	4641	-13145	21921
3.25	42780	52160	9380	8038	-20988	39748
5	42490	57487	14996	8038	-15372	45364
10	45613	34401	-11212	8038	-41580	19156
Biotest Sampler						
Overall	40716	7831	-32885	2257	-41411	-24339
3.25	38346	4021	-34325	3909	-49092	-19557
5	43930	13469	-30461	3909	-45228	-15693
10	39872	6004	-33869	3909	-48636	-19101
SAS Sampler						
Overall	42339	18197	-24142	2301	-32834	-15450
3.25	45481	14934	-30547	3985	-45601	-15492
5	33515	22278	-11237	3985	-26291	3818
10	48022	17379	-30643	3985	-45697	-15588

Note: p/m³ is particles per cubic meter of air.

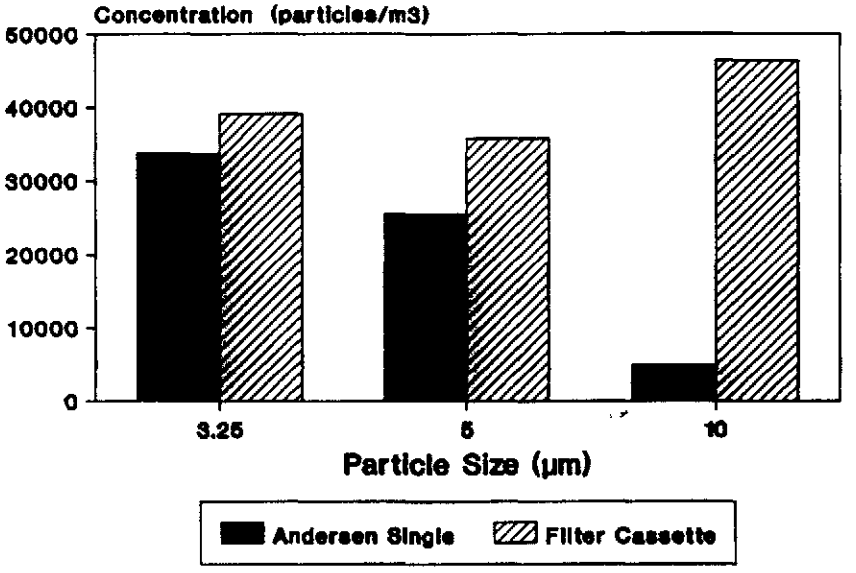


Figure 9. Summary Results of Andersen Single-stage Sampler Compared to Filter Cassette.

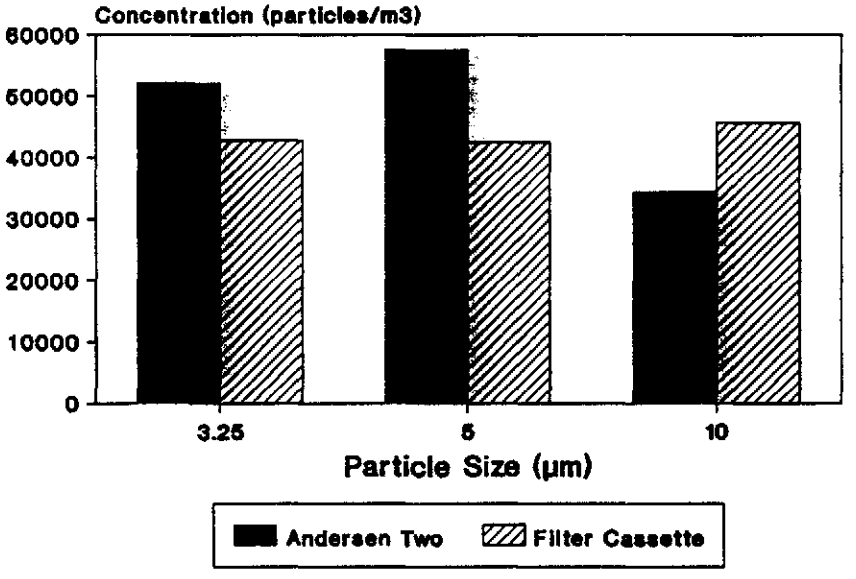


Figure 10. Summary Results of Andersen Two-stage Sampler Compared to Filter Cassette.

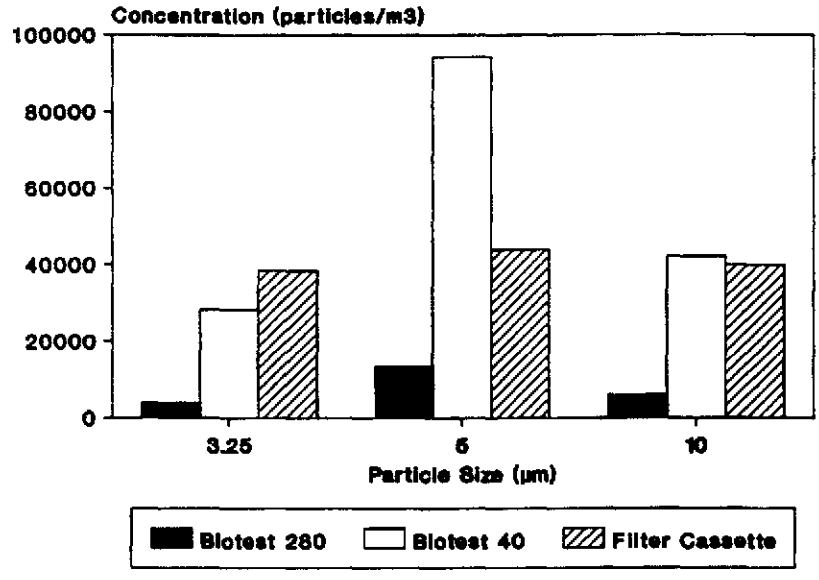


Figure 11. Summary Results of Biotest Sampler Compared to Filter Cassette.

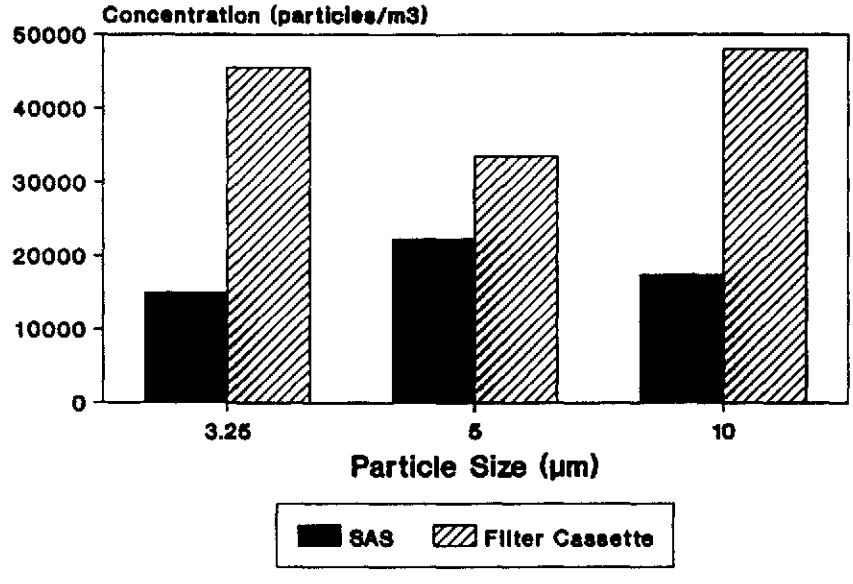


Figure 12. Summary Results of SAS Sampler Compared to Filter Cassette.

The results for the Andersen single- and two-stage samplers compared with the filter cassette are graphically summarized in Figures 9 and 10. For the Andersen single-stage sampler, overall bias was significant ($p<0.007$) and the variation of bias by particle size was significant ($p<0.025$). The mean bias was negative indicating that this sampler underestimates the reference sampler. The upper confidence level for this sampler at a particle size of 10 μm was negative indicating the sampler's inability to approach the reference sampler. This was not the case for the other particle size levels. Other analyses did not detect any pairwise differences among the three particle size ranges in amount of bias.

For the Andersen two-stage sampler, no detectable bias was found ($p<0.18$).

The results for the Biotest sampler compared with the filter cassette are graphically summarized in Figure 11. Note that two bars represent the Biotest particle concentration values, Biotest 280 and Biotest 40, based on concentration calculations using the total sampler flow rate of 280 lpm and the manufacturer's separation volumetric flow rate of 40 lpm, respectively. All statistical analyses are based on the values computed using the total sampler flow rate. For the Biotest sampler, the overall negative bias was significant ($p<0.0001$) indicating that this sampler underestimates relative to the reference sampler. There was no significant difference in bias among particle sizes ($p<0.76$). Observation of the Biotest 280 results indicate a good correlation with those obtained by Macher and First [1982] for the particle sizes of 3.25 and 5 μm. However, the concentration fall-off at 10 μm is inconsistent with the their results. This discrepancy at 10 μm may be due to the influence of the spiraling air outflow past the incoming air envelope and into the oncoming chamber airstream (and aerosol particles). Macher and First's results are not "tainted" by this effect due to the design of the sampling inlet. Observation of the Biotest 40 values in Figure 11 indicates close similarities between the sampler and the reference sampler at particle sizes of 3.25 and 10 μm and an extreme over-estimation at 5 μm. The discrepancies between the results of the Biotest 280 and Biotest 40 serves to emphasize the importance of proper flow rate selection. The use of the manufacturers sampler volumetric flow rate of 40 lpm may provide a good estimation of the "actual" ambient concentrations at the low and high ends of the respirable range, however, the excessive over-estimation of particle concentrations in the middle of the respirable range may preclude the use of this flow rate. The use of the total sampler flow rate of 280 lpm in the calculation of microbial concentration provides in a more accurate "picture" of the capabilities of the instrument).

The results for the SAS sampler compared with the filter cassette are graphically summarized in Figure 12. For the SAS sampler, overall bias was significant ($p<0.0002$) and the bias by particle size was significant ($p<0.021$). The upper confidence levels were negative with the exception of the particle size of 5 μm indicating the sampler's inability to approach the reference sampler at particle sizes of 3.25 and 10 μm. Other analyses indicate that particle sizes 3.25 and 10 μm do not have detectably different levels of bias but both differ from particle size of 5 μm.

The lack of statistical significance associated with sampler bias may be due to an inadequate amount of statistical power and may not be a direct indication of a samplers ability to approach the reference sampler. Power can be defined as the ability to detect a true alternative hypothesis.[32] For an ANOVA model having ***k*** treatments and ***n*** experimental units per treatment, the expected values of the mean squares for the overall F ratio are a function of variable termed the non-centrality parameter (δ). A simple ANOVA model for this study is given by:

$$Y_{ij} = \mu + \tau_i + e_{ij}$$

where μ is the mean bias, τ_i is the bias associated with the particle size effect, and e_{ij} is the error term. For δ equal to zero, the sampling distribution of the overall F ratio has a central F distribution F[(k-1),k(n-1)]. When δ is not equal to zero, the sampling distribution has a non-central F distribution F[(n-1),k(n-1);δ] dependent on the parameter δ. The non-centrality parameter can be determined by solving for δ in the following equation for the expected mean square for the hypothesis:[33]

$$E(MS_H) = \sigma^2 + q^{-1}\sigma^2\delta^2$$

where σ^2 is the variance of e_{ij} and q is the associated degrees of freedom.

For the simple model given above:

$$\delta = \sqrt{\frac{n}{\sigma^2}\sum_{i=1}^{3}(\mu+\tau_i)^2}$$

An approximation for the non-central F was used to determine percentile points required for the development of power curves. The approximation is based on the first two moments of the central F distribution and a forced commonality with the non-central F distribution.[34] A typical example of the power curves (various values of μ when τ=0) for the four samplers is shown in Figure 13.

Observation of the graph reveals acceptable statistical power for the SAS sampler and perhaps even the Biotest sampler. However, in this evaluation, the power of the test for the Andersen single- and two-stage samplers appears limited. This limited statistical power is probably related to the large values of the observed mean square error as compared to the mean square error for the other samplers. Although the

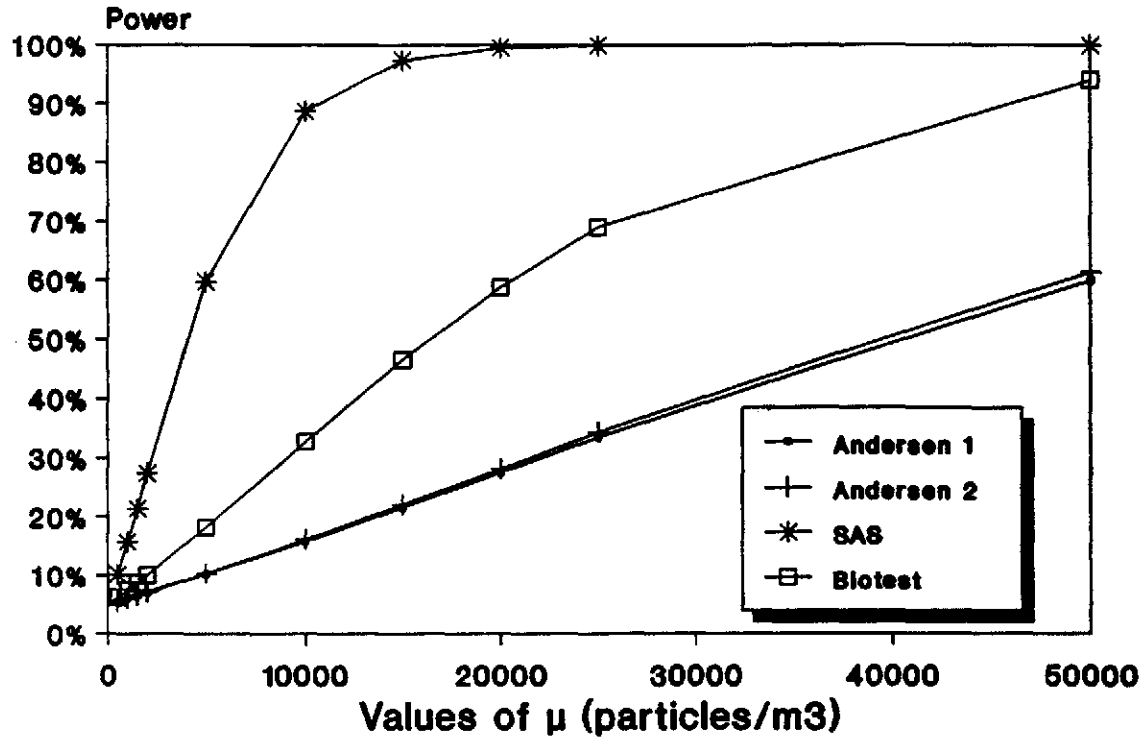


Figure 13. Typical Power Curves of the Simple ANOVA Model.

small sample sizes within experimental units reduced the power across all of the samplers, the effect was not uniform for comparisons between samplers.

B. COMPARISONS AMONG SAMPLERS

The existence of some relative bias is indicated regardless of whether the log or original scale response is analyzed ($p<0.0001$). Variations in relative bias among the samplers by particle size is detected with the log scale response but not the original scale response ($p<0.0508$). In both cases, differences in mean levels among samplers is significant. Non-simultaneous comparisons among the samplers by particle size levels are shown in Table 5 for the log scale data.

Observation of Table 5 for the Andersen single- and two-stage cascade impactors indicates no significant difference between the samplers except for the Andersen single-stage at a particle size of 10 μm which was significantly different compared to the Andersen two-stage at all particle size levels - an over-estimation of the two-stage to the single-stage. This contradicts the results of Gillespie et. al. [1981] which indicated an under-estimation of the Andersen two-stage to the single-stage (assuming the reliability of the results of Jones et. al. [1985] that the Andersen single-stage and

Table 5. Comparison of Sampler Measurements by Particle Size Level

SAMPLER	PARTICLE SIZE	LOG MEAN	LOG STD ERROR	NON-SIMULTANEOUS SIGNIFICANCE LEVELS FOR TESTING MEAN(I) = MEAN(J)											
				I/J	1	2	3	4	5	6	7	8	9	10	11
Andersen 1	3.25	10.27	0.211	1	•										
Andersen 1	5	10.14	0.211	2	0.6825	•									
Andersen 1	10	8.43	0.211	3	0.0001	0.0001	•								
Andersen 2	3.25	10.82	0.211	4	0.0756	0.0324	0.0001	•							
Andersen 2	5	10.96	0.211	5	0.0296	0.0116	0.0001	0.6545	•						
Andersen 2	10	10.39	0.211	6	0.6836	0.4166	0.0001	0.1613	0.0697	•					
Biotest	3.25	8.29	0.211	7	0.0001	0.0001	0.8288	0.0001	0.0001	0.0001	•				
Biotest	5	9.50	0.211	8	0.0167	0.0412	0.0016	0.0062	0.0001	0.0064	0.0006	•			
Biotest	10	8.70	0.211	9	0.0001	0.0001	0.3835	0.0001	0.0001	0.0001	0.1811	0.0134	•		
SAS	3.25	9.59	0.211	10	0.0337	0.0784	0.0007	0.0004	0.0001	0.0136	0.0002	0.7522	0.0064	•	
SAS	5	10.00	0.211	11	0.3786	0.6335	0.0001	0.0116	0.0036	0.2029	0.0001	0.1069	0.0002	0.1875	•
SAS	10	9.65	0.211	12	0.0504	0.1127	0.0004	0.0060	0.0002	0.2029	0.0001	0.5136	0.0040	0.8493	0.2561

NOTE: 1. In order to control the overall comparison error rate for multiple hypotheses, the Bonferroni procedure was used significance level of 0.00075.

2. Shaded areas indicate simultaneous statistical significance at the 0.05 level.

the six-stage results are comparable). The conclusions of Smid et. al. [1989] could not be duplicated for the SAS and the Andersen single-stage samplers (i.e. the SAS undersampled the Andersen sampler by approximately 50%). Table 5 only indicates statistical significance for the Andersen single-stage sampler at a particle size of 10 μm compared to the SAS at all particle size levels - an over-estimation of the SAS to the Andersen sampler. However, the results of the SAS sampler, when assessed alone, exhibits an overall mean bias of -57% to the reference sampler which does correlate with Smid's conclusions. Nakhla and Cummings [1981] observed an over-estimation of the Biotest sampler compared to the SAS sampler in a hospital environment which contradicts the results listed in Table 5 (which indicate a general tendency for the Biotest to under-estimate the SAS sampler). This may be due, in part, to Nakhla and Cummings' use of the manufacturer's separation volumetric flow rate value of 40 lpm in the calculation of the microbial concentrations as opposed to the total sampler flow rate of 280 lpm. The results of these documented comparison studies are based on readings taken under field conditions, hence, the variability of the microorganisms sampled, the general size distribution of the aerosols sampled, and the conditions of the ambient environment complicate their comparability with this study.

Kang and Frank [1989] assessed sampler efficiencies (including the AGI-30, membrane filter, Biotest, and Andersen six-stage cascade impactor) under controlled experimental conditions in an aerosol chamber. An evaluation in the laboratory facilitated the elimination of the variability experienced in the field evaluations due to the general size distribution of the aerosols sampled and the conditions of the ambient environment. Their results for the Biotest and the Andersen six-stage (again, assuming Jones' et. al. comparability of the Andersen six- and single-stage cascade impactors) exhibit a fair correlation with the results of Table 5 for particles in the lower portion of the respirable range.

The objectives of the study did not include analysis of the precision of the samplers, however, some information is available. Estimates of the coefficients of variation (CV) are listed in the following table:

Table 6. Estimates of Coefficients of Variation

SAMPLER	COEFFICIENT OF VARIATION
Andersen Single-Stage	67%
Andersen Two-Stage	29%
Biotest	18%
SAS	32%
Filter Cassette	14%

The study CV for the Biotest sampler correlates fairly well with the documented work of Kang and Frank which encompasses a range of 17-33%, of Placencia et. al. [1982] which encompasses a range of 13-33%, and Nakhla and Cummings which encompasses a range of 11-53%. However, the study Biotest CV is slightly below those values observed by Smid et. al. which had a range of 24-37%. The study CV for the SAS sampler falls within the range of 24-82% documented by Nakhla and Cummings. Smid et. al. reports a CV range of 29-40% for the Andersen single-stage sampler - this is below the observed CV for this study. However, the study CV for the Andersen two-stage sampler falls within this range. Due to the tight similarities between the Andersen single- and two-stage samplers (i.e. instrument design, sampling methodologies, and analytical techniques), it would not be an unreasonable assumption to expect the CV values to be relatively close. The lack of consistency between the CV values for the Andersen samplers appears unknown.

VI. CONCLUSIONS

The results of the microbial surrogate aerosol phase of the study indicates that the Andersen two-stage sampler differs from the other samplers except for the reference sampler, the filter cassette, in terms of the level of measurement on the average and by particle size level. Although the Andersen two-stage sampler over sampled the reference sampler, overall, by a bias of 10%, it was not statistically significant.

The Andersen single-stage sampler under sampled the reference sampler by 47%, which was a significant difference. For a particle size of 3.25 μm, this sampler under sampled the reference sampler by -10%. Subsequently, at increasing particles sizes of 5 and 10 μm, the sampling efficiency began to fall off with biases of -29% and -89%, respectively. In addition, the observed CV for the Andersen single-stage was the highest of all the samplers at 67% possibly indicating a certain amount of imprecision in this instrument.

The Biotest and the SAS sampler both under sampled the reference sampler by -81% and -57%, respectively. Both of these biases were statistically significant. However, it is important to note that for the Biotest sampler the researchers used the total sampling flow rate of the instrument (280 lpm) was used in the computations and not the manufacturers recommended "separation" flow rate of 40 lpm. There was not a statistical difference among particle size ranges for the bias of the Biotest sampler. However, there was a statistical difference among particle size ranges for the bias of the SAS sampler. Specifically, the sampling efficiency of the instrument fell off to -67% and -64% at 3.25 and 10 μm, and respectively, from -34% at a particle size of 5 μm.

Based on these results, if the particle size distribution of a proposed sampling location is unknown or has a varied range similar to that of this study, it appears that the Andersen two-stage sampler offers the most reliable results. However, this conclusion must be tempered by the knowledge that the power for this sampler to detect bias was relatively low and, overall, only the effects of particle size on bias were studied.

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APPENDIX

SAMPLER	SAMPLER STAGE	PLATE NO.	FLOWRATE (lpm)	DATE	TIME	PARTICLE SIZE	PARTICLE CONCENTRATION (p/m3)
BIOTEST		B1B	280	05-May	10:30	5.00	9203
BIOTEST		B2B	280	05-May	10:40	5.00	11920
BIOTEST		B3B	280	05-May	10:49	5.00	12141
SAS		S1B	90	05-May	11:02	5.00	22275
SAS		S2B	90	05-May	11:08	5.00	22815
SAS		S4B	90	05-May	11:15	5.00	7262
FILTER CASSETTE - 37 MM		FC1B	20	05-May	10:30	5.00	6408
FILTER CASSETTE - 37 MM		FC2B	20	05-May	10:40	5.00	48252
FILTER CASSETTE - 37 MM		FC3B	20	05-May	10:49	5.00	36819
FILTER CASSETTE - 37 MM		FC5B	20	05-May	11:02	5.00	44411
FILTER CASSETTE - 37 MM		FC6B	20	05-May	11:08	5.00	36441
FILTER CASSETTE - 37 MM		FC8B	20	05-May	11:15	5.00	11771
BIOTEST		B5C	280	08-May	14:58	5.00	15011
BIOTEST		B6C	280	08-May	15:08	5.00	16413
BIOTEST		B7C	280	08-May	15:18	5.00	16168
ANDERSEN SINGLE STAGE		AS5C	28	08-May	13:57	5.00	27211
ANDERSEN SINGLE STAGE		AS6C	28	08-May	14:15	5.00	28415
ANDERSEN SINGLE STAGE		AS7C	28	08-May	14:35	5.00	31147
FILTER CASSETTE - 37 MM		FC8C	20	08-May	13:57	5.00	48111
FILTER CASSETTE - 37 MM		FC9C	20	08-May	14:15	5.00	37162
FILTER CASSETTE - 37 MM		FC10C	20	08-May	14:35	5.00	35458
FILTER CASSETTE - 37 MM		FC12C	20	08-May	14:58	5.00	52798
FILTER CASSETTE - 37 MM		FC13C	20	08-May	15:08	5.00	52152
FILTER CASSETTE - 37 MM		FC14C	20	08-May	15:20	5.00	52152
ANDERSEN TWO-STAGE	1	AT1D	28	10-May	09:34	5.00	4909
ANDERSEN TWO-STAGE	2	AT2D	28	10-May	09:34	5.00	57763
ANDERSEN TWO-STAGE	1	AT4D	28	10-May	09:54	5.00	3349
ANDERSEN TWO-STAGE	2	AT3D	28	10-May	09:54	5.00	53729
ANDERSEN TWO-STAGE	1	AT9D	28	10-May	10:37	5.00	4210
ANDERSEN TWO-STAGE	2	AT10D	28	10-May	10:37	5.00	40931
ANDERSEN SINGLE STAGE		AS1D	28	10-May	10:55	5.00	20092
ANDERSEN SINGLE STAGE		AS2D	28	10-May	11:15	5.00	21349
ANDERSEN SINGLE STAGE		AS3D	28	10-May	11:35	5.00	28912
FILTER CASSETTE - 37 MM		FC1D	20	10-May	09:34	5.00	36885
FILTER CASSETTE - 37 MM		FC2D	20	10-May	09:54	5.00	32604
FILTER CASSETTE - 37 MM		FC8D	20	10-May	10:37	5.00	37510

SAMPLER	SAMPLER STAGE	PLATE NO.	FLOWRATE (lpm)	DATE	TIME	PARTICLE SIZE	PARTICLE CONCENTRATION (p/m3)
FILTER CASSETTE - 37 MM		FC5D	20	10-May	10:55	5.00	24175
FILTER CASSETTE - 37 MM		FC6D	20	10-May	11:15	5.00	16865
FILTER CASSETTE - 37 MM		FC7D	20	10-May	11:35	5.00	47685
ANDERSEN SINGLE STAGE		AS4E	28	11-May	09:45	5.00	20980
ANDERSEN SINGLE STAGE		AS1E	28	11-May	10:03	5.00	23943
ANDERSEN SINGLE STAGE		AS5E	28	11-May	11:09	5.00	27541
SAS		S2E	90	11-May	10:47	5.00	23387
SAS		S5E	90	11-May	10:54	5.00	23010
SAS		S3E	90	11-May	11:00	5.00	25095
FILTER CASSETTE - 37 MM		FC1E	20	11-May	09:45	5.00	37367
FILTER CASSETTE - 37 MM		FC2E	20	11-May	10:03	5.00	31410
FILTER CASSETTE - 37 MM		FC5E	20	11-May	10:47	5.00	40423
FILTER CASSETTE - 37 MM		FC6E	20	11-May	10:54	5.00	42220
FILTER CASSETTE - 37 MM		FC7E	20	11-May	11:00	5.00	41122
FILTER CASSETTE - 37 MM		FC8E	20	11-May	11:09	5.00	44056
BIOTEST		B1F	280	16-May	09:16	5.00	12433
BIOTEST		B2F	280	16-May	09:23	5.00	13722
BIOTEST		B4F	280	16-May	09:37	5.00	14214
ANDERSEN TWO-STAGE	1	AT1F	28	16-May	09:51	5.00	13822
ANDERSEN TWO-STAGE	2	AT2F	28	16-May	09:51	5.00	52084
ANDERSEN TWO-STAGE	1	AT6F	28	16-May	10:25	5.00	6573
ANDERSEN TWO-STAGE	2	AT5F	28	16-May	10:25	5.00	53516
ANDERSEN TWO-STAGE	1	AT3F	28	16-May	10:50	5.00	5051
ANDERSEN TWO-STAGE	2	AT4F	28	16-May	10:50	5.00	59245
FILTER CASSETTE - 37 MM		FC1F	20	16-May	09:16	5.00	47307
FILTER CASSETTE - 37 MM		FC2F	20	16-May	09:23	5.00	47782
FILTER CASSETTE - 37 MM		FC3F	20	16-May	09:37	5.00	51700
FILTER CASSETTE - 37 MM		FC5F	20	16-May	09:51	5.00	47710
FILTER CASSETTE - 37 MM		FC6F	20	16-May	10:25	5.00	50433
FILTER CASSETTE - 37 MM		FC7F	20	16-May	10:50	5.00	48090
ANDERSEN TWO-STAGE	1	AT2G	28	18-May	10:04	5.00	4010
ANDERSEN TWO-STAGE	2	AT3G	28	18-May	10:04	5.00	52545
ANDERSEN TWO-STAGE	1	AT4G	28	18-May	10:23	5.00	3922
ANDERSEN TWO-STAGE	2	AT5G	28	18-May	10:23	5.00	51227
ANDERSEN TWO-STAGE	1	AT6G	28	18-May	10:42	5.00	2736
ANDERSEN TWO-STAGE	2	AT7G	28	18-May	10:42	5.00	47757

SAMPLER	SAMPLER STAGE	PLATE NO.	FLOWRATE (lpm)	DATE	TIME	PARTICLE SIZE	PARTICLE CONCENTRATION (p/m3)
SAS		S2G	90	18-May	09:39	5.00	26161
SAS		S1G	90	18-May	09:44	5.00	25042
SAS		S3G	90	18-May	09:54	5.00	25457
FILTER CASSETTE - 37 MM		FC1G	20	18-May	09:39	5.00	40990
FILTER CASSETTE - 37 MM		FC2G	20	18-May	09:44	5.00	52177
FILTER CASSETTE - 37 MM		FC3G	20	18-May	09:54	5.00	43227
FILTER CASSETTE - 37 MM		FC5G	20	18-May	10:04	5.00	42004
FILTER CASSETTE - 37 MM		FC6G	20	18-May	10:23	5.00	44304
FILTER CASSETTE - 37 MM		FC7G	20	18-May	10:42	5.00	42874
ANDERSEN SINGLE STAGE		AS8H	28	13-Jun	09:07	10.00	6461
ANDERSEN SINGLE STAGE		AS4H	28	13-Jun	09:26	10.00	6952
ANDERSEN SINGLE STAGE		AS7H	28	13-Jun	09:45	10.00	7574
ANDERSEN SINGLE STAGE		AS5H	28	13-Jun	13:34	3.25	64566
ANDERSEN SINGLE STAGE		AS6H	28	13-Jun	13:52	3.25	59226
ANDERSEN SINGLE STAGE		AS1H	28	13-Jun	14:10	3.25	61920
SAS		S4H	90	13-Jun	10:05	10.00	22513
SAS		S2H	90	13-Jun	10:12	10.00	22729
SAS		S8H	90	13-Jun	10:20	10.00	21216
SAS		S6H	90	13-Jun	13:08	3.25	19618
SAS		S1H	90	13-Jun	13:15	3.25	16829
SAS		S5H	90	13-Jun	13:22	3.25	19731
FILTER CASSETTE - 37 MM		FC5H	20	13-Jun	10:05	3.25	29747
FILTER CASSETTE - 37 MM		FC14H	20	13-Jun	14:10	3.25	50671
FILTER CASSETTE - 37 MM		FC12H	20	13-Jun	13:34	3.25	48241
FILTER CASSETTE - 37 MM		FC13H	20	13-Jun	13:52	3.25	30057
FILTER CASSETTE - 37 MM		FC9H	20	13-Jun	13:15	3.25	49990
FILTER CASSETTE - 37 MM		FC3H	20	13-Jun	09:45	10.00	52714
FILTER CASSETTE - 37 MM		FC1H	20	13-Jun	09:07	10.00	52436
FILTER CASSETTE - 37 MM		FC6H	20	13-Jun	10:12	10.00	59190
FILTER CASSETTE - 37 MM		FC8H	20	13-Jun	13:08	3.25	45334
FILTER CASSETTE - 37 MM		FC10H	20	13-Jun	13:22	3.25	37541
FILTER CASSETTE - 37 MM		FC2H	20	13-Jun	09:26	10.00	45996
FILTER CASSETTE - 37 MM		FC7H	20	13-Jun	10:20	10.00	48210
ANDERSEN TWO-STAGE	1	AT1I	28	15-Jun	09:05	10.00	54999
ANDERSEN TWO-STAGE	2	AT2I	28	15-Jun	09:05	10.00	741
ANDERSEN TWO-STAGE	1	AT4I	28	15-Jun	09:25	10.00	39366

SAMPLER	SAMPLER STAGE	PLATE NO.	FLOWRATE (lpm)	DATE	TIME	PARTICLE SIZE	PARTICLE CONCENTRATION (p/m3)
ANDERSEN TWO-STAGE	2	AT5I	28	15-Jun	09:25	10.00	5736
ANDERSEN TWO-STAGE	1	AT6I	28	15-Jun	09:44	10.00	53093
ANDERSEN TWO-STAGE	2	AT7I	28	15-Jun	09:44	10.00	589
SAS		S7I	90	15-Jun	10:12	10.00	25829
SAS		S8I	90	15-Jun	10:19	10.00	15937
SAS		S5I	90	15-Jun	10:26	10.00	25613
SAS		S6I	90	15-Jun	13:31	3.25	24568
SAS		S4I	90	15-Jun	13:38	3.25	13697
SAS		S1I	90	15-Jun	13:50	3.25	4713
ANDERSEN TWO-STAGE	1	AT8I	28	15-Jun	13:58	3.25	819
ANDERSEN TWO-STAGE	2	AT9I	28	15-Jun	13:58	3.25	70268
ANDERSEN TWO-STAGE	1	AT10I	28	15-Jun	14:17	3.25	570
ANDERSEN TWO-STAGE	2	AT11I	28	15-Jun	14:17	3.25	71989
ANDERSEN TWO-STAGE	1	AT13I	28	15-Jun	14:36	3.25	292
ANDERSEN TWO-STAGE	2	AT14I	28	15-Jun	14:36	3.25	70517
FILTER CASSETTE - 37 MM		FC1I	20	15-Jun	09:05	10.00	42826
FILTER CASSETTE - 37 MM		FC2I	20	15-Jun	09:25	10.00	47681
FILTER CASSETTE - 37 MM		FC3I	20	15-Jun	09:44	10.00	49354
FILTER CASSETTE - 37 MM		FC6I	20	15-Jun	10:12	10.00	57098
FILTER CASSETTE - 37 MM		FC7I	20	15-Jun	10:19	10.00	51271
FILTER CASSETTE - 37 MM		FC8I	20	15-Jun	10:36	10.00	48519
FILTER CASSETTE - 37 MM		FC10I	20	15-Jun	13:31	3.25	59315
FILTER CASSETTE - 37 MM		FC11I	20	15-Jun	13:38	3.25	52832
FILTER CASSETTE - 37 MM		FC12I	20	15-Jun	13:50	3.25	39984
FILTER CASSETTE - 37 MM		FC14I	20	15-Jun	13:58	3.25	40479
FILTER CASSETTE - 37 MM		FC15I	20	15-Jun	14:17	3.25	52659
FILTER CASSETTE - 37 MM		FC16I	20	15-Jun	14:36	3.25	37414
BIOTEST		B3J	280	20-Jun	09:13	3.25	2838
BIOTEST		B4J	280	20-Jun	09:24	3.25	3328
BIOTEST		B1J	280	20-Jun	09:35	3.25	3486
ANDERSEN SINGLE STAGE		AS4J	28	20-Jun	09:59	3.25	15227
ANDERSEN SINGLE STAGE		AS5J	28	20-Jun	10:18	3.25	17695
ANDERSEN SINGLE STAGE		AS7J	28	20-Jun	10:35	3.25	19643
ANDERSEN SINGLE STAGE		AS1J	28	20-Jun	13:33	10.00	5406
ANDERSEN SINGLE STAGE		AS6J	28	20-Jun	13:52	10.00	5281
ANDERSEN SINGLE STAGE		AS3J	28	20-Jun	14:10	10.00	6090

SAMPLER	SAMPLER STAGE	PLATE NO.	FLOWRATE (lpm)	DATE	TIME	PARTICLE SIZE	PARTICLE CONCENTRATION (p/m3)
BIOTEST		B6J	280	20-Jun	14:31	10.00	6073
BIOTEST		B8J	280	20-Jun	14:41	10.00	5860
BIOTEST		B5J	280	20-Jun	14:51	10.00	5937
FILTER CASSETTE - 37 MM		FC1J	20	20-Jun	09:13	3.25	25294
FILTER CASSETTE - 37 MM		FC2J	20	20-Jun	09:24	3.25	38331
FILTER CASSETTE - 37 MM		FC3J	20	20-Jun	09:35	3.25	29560
FILTER CASSETTE - 37 MM		FC5J	20	20-Jun	09:59	3.25	35567
FILTER CASSETTE - 37 MM		FC6J	20	20-Jun	10:18	3.25	40565
FILTER CASSETTE - 37 MM		FC7J	20	20-Jun	10:35	3.25	35343
FILTER CASSETTE - 37 MM		FC8J	20	20-Jun	13:33	10.00	41233
FILTER CASSETTE - 37 MM		FC9J	20	20-Jun	13:52	10.00	47744
FILTER CASSETTE - 37 MM		FC10J	20	20-Jun	14:10	10.00	38021
FILTER CASSETTE - 37 MM		FC12J	20	20-Jun	14:31	10.00	38869
FILTER CASSETTE - 37 MM		FC13J	20	20-Jun	14:41	10.00	34084
FILTER CASSETTE - 37 MM		FC14J	20	20-Jun	14:51	10.00	41096
BIOTEST		B7K	280	22-Jun	13:19	3.25	4035
BIOTEST		B1K	280	22-Jun	13:29	3.25	4163
BIOTEST		B8K	280	22-Jun	13:39	3.25	4807
SAS		S8K	90	22-Jun	13:50	3.25	5962
SAS		S3K	90	22-Jun	13:57	3.25	17468
SAS		S6K	90	22-Jun	14:04	3.25	11817
SAS		S1K	90	22-Jun	09:22	10.00	10257
SAS		S7K	90	22-Jun	09:29	10.00	7960
SAS		S2K	90	22-Jun	09:36	10.00	4360
BIOTEST		B2K	280	22-Jun	09:46	10.00	5658
BIOTEST		B3K	280	22-Jun	09:52	10.00	6094
BIOTEST		B4K	280	22-Jun	10:07	10.00	6493
FILTER CASSETTE - 37 MM		FC1K	20	22-Jun	09:22	10.00	49639
FILTER CASSETTE - 37 MM		FC2K	20	22-Jun	09:29	10.00	44472
FILTER CASSETTE - 37 MM		FC3K	20	22-Jun	09:36	10.00	44053
FILTER CASSETTE - 37 MM		FC5K	20	22-Jun	09:46	10.00	38445
FILTER CASSETTE - 37 MM		FC6K	20	22-Jun	09:52	10.00	47436
FILTER CASSETTE - 37 MM		FC7K	20	22-Jun	10:07	10.00	45166
FILTER CASSETTE - 37 MM		FC8K	20	22-Jun	13:19	3.25	44593
FILTER CASSETTE - 37 MM		FC9K	20	22-Jun	13:29	3.25	36709
FILTER CASSETTE - 37 MM		FC10K	20	22-Jun	13:39	3.25	38680

SAMPLER	SAMPLER STAGE	PLATE NO.	FLOWRATE (lpm)	DATE	TIME	PARTICLE SIZE	PARTICLE CONCENTRATION (p/m3)
FILTER CASSETTE - 37 MM		FC12K	20	22-Jun	13:57	3.25	42460
FILTER CASSETTE - 37 MM		FC13K	20	22-Jun	14:04	3.25	44291
FILTER CASSETTE - 37 MM		FC14K	20	22-Jun	14:51	3.25	37578
ANDERSEN TWO-STAGE	2	AT10L	28	26-Jun	09:36	10.00	0
ANDERSEN TWO-STAGE	1	AT11L	28	26-Jun	09:36	10.00	24501
ANDERSEN TWO-STAGE	1	AT13L	28	26-Jun	09:56	10.00	18386
ANDERSEN TWO-STAGE	2	AT14L	28	26-Jun	09:56	10.00	0
ANDERSEN TWO-STAGE	1	AT8L	28	26-Jun	10:16	10.00	31862
ANDERSEN TWO-STAGE	2	AT9L	28	26-Jun	10:16	10.00	0
BIOTEST		B3L	280	26-Jun	10:35	10.00	5878
BIOTEST		B6L	280	26-Jun	10:45	10.00	5947
BIOTEST		B7L	280	26-Jun	10:55	10.00	6092
BIOTEST		B5L	280	26-Jun	13:29	3.25	4129
BIOTEST		B2L	280	26-Jun	13:39	3.25	4903
BIOTEST		B1L	280	26-Jun	13:49	3.25	4504
ANDERSEN TWO-STAGE	1	AT3L	28	26-Jun	14:18	3.25	0
ANDERSEN TWO-STAGE	2	AT4L	28	26-Jun	14:18	3.25	25501
ANDERSEN TWO-STAGE	1	AT5L	28	26-Jun	14:38	3.25	0
ANDERSEN TWO-STAGE	2	AT6L	28	26-Jun	14:38	3.25	41707
ANDERSEN TWO-STAGE	1	AT1L	28	26-Jun	14:58	3.25	0
ANDERSEN TWO-STAGE	2	AT2L	28	26-Jun	14:58	3.25	39150
FILTER CASSETTE - 37 MM		FC1L	20	26-Jun	09:36	10.00	36301
FILTER CASSETTE - 37 MM		FC2L	20	26-Jun	09:56	10.00	50282
FILTER CASSETTE - 37 MM		FC3L	20	26-Jun	10:16	10.00	42751
FILTER CASSETTE - 37 MM		FC5L	20	26-Jun	10:35	10.00	29906
FILTER CASSETTE - 37 MM		FC6L	20	26-Jun	10:45	10.00	42600
FILTER CASSETTE - 37 MM		FC7L	20	26-Jun	10:55	10.00	41248
FILTER CASSETTE - 37 MM		FC8L	20	26-Jun	13:29	3.25	43636
FILTER CASSETTE - 37 MM		FC9L	20	26-Jun	13:39	3.25	46809
FILTER CASSETTE - 37 MM		FC10L	20	26-Jun	13:49	3.25	41503
FILTER CASSETTE - 37 MM		FC12L	20	26-Jun	14:18	3.25	48801
FILTER CASSETTE - 37 MM		FC13L	20	26-Jun	14:38	3.25	43082
FILTER CASSETTE - 37 MM		FC14L	20	26-Jun	14:58	3.25	45621
ANDERSEN TWO-STAGE	1	AT2M	28	27-Jun	09:24	10.00	43020
ANDERSEN TWO-STAGE	2	AT3M	28	27-Jun	09:24	10.00	0
ANDERSEN TWO-STAGE	1	AT4M	28	27-Jun	09:42	10.00	16681

SAMPLER	SAMPLER STAGE	PLATE NO.	FLOWRATE (lpm)	DATE	TIME	PARTICLE SIZE	PARTICLE CONCENTRATION (p/m3)
ANDERSEN TWO-STAGE	2	AT5M	28	27-Jun	09:42	10.00	159
ANDERSEN TWO-STAGE	1	AT6M	28	27-Jun	09:59	10.00	20476
ANDERSEN TWO-STAGE	2	AT7M	28	27-Jun	09:59	10.00	0
ANDERSEN SINGLE STAGE		AS8M	28	27-Jun	10:20	10.00	3322
ANDERSEN SINGLE STAGE		AS5M	28	27-Jun	10:39	10.00	2010
ANDERSEN SINGLE STAGE		AS7M	28	27-Jun	10:56	10.00	2153
ANDERSEN SINGLE STAGE		AS6M	28	27-Jun	13:19	3.25	17351
ANDERSEN SINGLE STAGE		AS1M	28	27-Jun	13:36	3.25	22027
ANDERSEN SINGLE STAGE		AS3M	28	27-Jun	13:56	3.25	26308
ANDERSEN TWO-STAGE	1	AT11M	28	27-Jun	14:13	3.25	1745
ANDERSEN TWO-STAGE	2	AT12M	28	27-Jun	14:13	3.25	32149
ANDERSEN TWO-STAGE	1	AT8M	28	27-Jun	14:32	3.25	1164
ANDERSEN TWO-STAGE	2	AT9M	28	27-Jun	14:32	3.25	49668
ANDERSEN TWO-STAGE	1	AT13M	28	27-Jun	14:49	3.25	1642
ANDERSEN TWO-STAGE	2	AT14M	28	27-Jun	14:49	3.25	62261
FILTER CASSETTE - 37 MM		FC1M	20	27-Jun	09:24	10.00	39584
FILTER CASSETTE - 37 MM		FC2M	20	27-Jun	09:42	10.00	56744
FILTER CASSETTE - 37 MM		FC3M	20	27-Jun	09:59	10.00	44994
FILTER CASSETTE - 37 MM		FC5M	20	27-Jun	10:20	10.00	48709
FILTER CASSETTE - 37 MM		FC6M	20	27-Jun	10:39	10.00	46569
FILTER CASSETTE - 37 MM		FC7M	20	27-Jun	10:56	10.00	44187
FILTER CASSETTE - 37 MM		FC8M	20	27-Jun	13:19	3.25	37931
FILTER CASSETTE - 37 MM		FC9M	20	27-Jun	13:36	3.25	48782
FILTER CASSETTE - 37 MM		FC10M	20	27-Jun	13:56	3.25	24786
FILTER CASSETTE - 37 MM		FC12M	20	27-Jun	14:13	3.25	55192
FILTER CASSETTE - 37 MM		FC13M	20	27-Jun	14:32	3.25	23404
FILTER CASSETTE - 37 MM		FC14M	20	27-Jun	14:49	3.25	38372