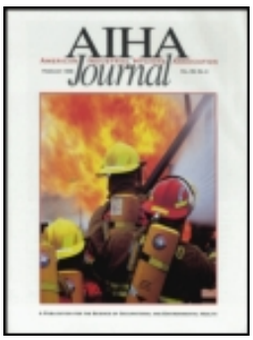

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Development and Evaluation of an Inhalable Bioaerosol Manifold Sampler

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This paper describes the development and evaluation of an inhalable bioaerosol manifold sampler for the collection of replicate samples over sequential sampling periods. Wind tunnel experiments at wind velocities ≤0.6 m/sec (≤~120 ft/min) and at a sampling velocity of 0.6 m/sec yielded sampling efficiencies within 10% of the American Conference of Governmental Industrial Hygienists' inhalable curve up to particle diameters of about 18 µm and efficiencies within 18% for diameters up to 26 µm. At wind velocities ≥0.6 m/sec, a sampling velocity of 0.8 m/sec yielded efficiencies that were within 15% of the inhalable curve up to diameters of about 22 µm and within 20% for diameters up to 25 µm. Microscopic counts of 15.6-µm and 25.6-µm monodisperse polystyrene beads aerosolized in the wind tunnel and collected with the manifold sampler yielded precise concentrations (pooled CV=9.0 and 5.3%, respectively) averaging 115% and 92%, respectively, of measurements with the APS Model 3310 aerodynamic particle sizer. Field tests in a cotton dust environment demonstrated the ability to generate a time/concentration profile of fungal spores similar to that indicated for cotton dust levels determined with horizontal elutriators. Cotton dust was sampled efficiently while cotton fibers were excluded. Field tests on a dairy farm indicated no statistically significant bias (p=0.72) between spore concentrations determined with the manifold sampler and concentrations determined through a central test port.

Keywords: aerosol sampler, bioaerosol, inhalable size fraction, microscopy, sampling

Considerable evidence exists linking the inhalation of microorganisms, their propagules, and chemicals produced by microorganisms to acute and chronic health effects.[1–13] Although culturable count concentrations of fungal spores and bacteria in air samples are the measures most frequently taken in environments suspected of being contaminated with biogenic substances, several difficulties arise when relating symptoms to such measures. Not only are airborne viable microbial concentrations subject to sampling and cultivation bias, viable counts are inadequate measures of the inflammatory and allergenic potential of biogenic dust because these responses can be induced by nonviable cells that may be present in far higher concentrations than viable cells.[14] A more appropriate measure of inflammatory and allergenic potential, especially in agricultural environments, is the microscopic enumeration of total (viable and nonviable) bacteria and fungal spores collected from the air onto filters.[15,16] The use of filter discs for collection of bioaerosols allows considerable flexibility in exposure averaging times and also in analysis, including microscopy and the determination of biochemicals such as bacterial endotoxin, (1→3)-β-D-glucans, and mycotoxins.[17,18]

Sequential sampling with filters in a manifold system offers the ability to track changes in bioaerosol concentrations over small time intervals and, conversely, the ability to determine time-weighted averages over extended time intervals. When determining the effects of variable environmental conditions or employee activity on

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bioaerosol levels, the high temporal variability of air spora in indoor and outdoor environments, as pointed out by Flannigan and Miller,[5] requires the determination of concentration-time profiles. Variations of 3 to 4 orders of magnitude have been observed over 1-hr intervals.[19] On the other hand, when estimating exposure to highly fluctuating concentrations of bioaerosols, sampling times must be long enough to represent the average exposure level, or short sequential samples must be combined to obtain the average level. To sample sequentially with viable samplers is usually cost prohibitive because many samples have to be taken. Sampling times with most viable samplers are restricted to small intervals (3 to 5 min) resulting in only "snapshot" concentrations.

Vincent[20] has pointed out that criteria for aerosol exposure guidelines should be specified in terms of all that is inhaled, and thus the ability to measure the inhalable fraction of aerosols is an important objective in many occupational situations. The importance of inhalability was first recognized by Ogden and Birkett in 1977.[21] These investigators demonstrated that the efficiency with which aerosols entered the nose and mouth of a life-size model of the human head and shoulders in a wind tunnel varied by 10-fold at different orientations. However, when averages were taken for all wind directions to simulate a worker randomly oriented to the wind, the efficiencies observed for wind velocities of 1–3 m/sec lay within a fairly small range for a given particle size. Vincent and Armbruster[22] later summarized these findings and those of other studies.[23,24] The American Conference of Governmental Industrial Hygienists (ACGIH) has adopted the following mathematical definition of inhalable penetration (I) as a function of aerodynamic equivalent diameter (d_{ae}):[25]

$$I = 0.5[1+\exp(-0.06\, d_{ae})] \text{ for } 0 < d_{ae} < 100\ \mu m \qquad (1)$$

At present, the Institute of Occupational Medicine (IOM) area inhalable aerosol sampler[26] is the only commercially available device specifically designed for the fixed-point collection of the inhalable fraction as defined above.[20] However, the device is primarily designed for the gravimetric determination of the collected particles and specifically does not provide uniform deposition of particles on the filter collection surface as required for microscopy.[26]

This article describes the development and evaluation of the performance of an inhalable bioaerosol manifold sampler. The device was designed to provide the following features: (1) flexibility in the choice of the aerosol sizing characteristics of the sampler, including the selective sampling of the inhalable fraction of aerosols; (2) collection efficiencies that are independent of wind speed and direction; (3) the ability to sample sequentially with a sampling manifold to develop time-concentration profiles over extended periods; and (4) the ability to use filter discs for collection of bioaerosols to allow flexibility in analysis, particularly the on-site enumeration of fungal propagules and bacteria by microscopy.

MATERIALS AND METHODS

Description of the Sampling Device—The Manifold Sampler

Using the design criteria set forth in the introduction, the following sampling device has been fabricated. A side-view drawing of the manifold sampler is presented in Figure 1, and a top view of the manifold is presented in Figure 2. The sampling manifold allows the collection of replicate samples over 6 sequential sampling periods with 12 small stainless steel sample holders situated in pairs around a ring. To minimize the effects of wind direction

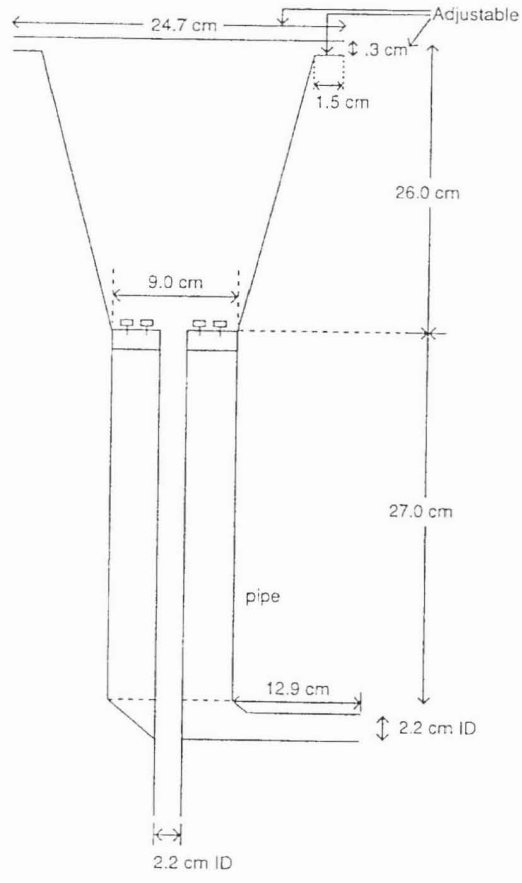

FIGURE 1. Schematic drawing of vertical cross section of manifold sampler

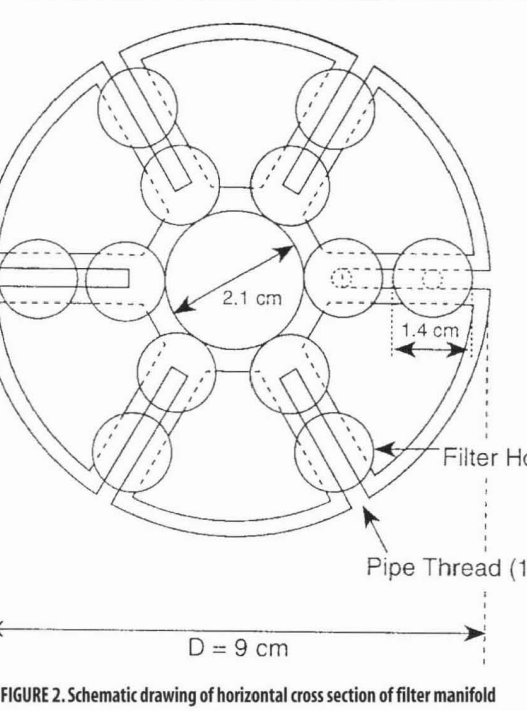

FIGURE 2. Schematic drawing of horizontal cross section of filter manifold

and velocity, the inlet of the manifold sampler is patterned after the flanged inlet developed by Liu and Pui for ambient air sampling.[27] The flange straightens the streamlines in the inlet and minimizes external impaction losses, and the large diameter of the inlet causes the streamlines to bend gradually thereby minimizing internal impaction losses.[28] The circumference of the inlet is 73 cm. In the depicted orientation, the flange and the inner surface of the cone contribute to the elutriation of large particles and are coated with silicone grease to prevent reentrainment of the collected aerosol. The 27° taper of the cone (with respect to vertical) helps to stabilize the velocity front as it moves toward the manifold. Both the flange and the lid of the sampler are machined to provide a knife-edge at the inlet slit. The width of the slit is adjustable but has been currently set at a height of 0.3 cm for laboratory and field evaluation.

Each filter holder in the sampling manifold is fabricated from a Millipore Swinney stainless steel holder for 13-mm filters (Millipore Corp., Bedford, Mass., catalog no. XX30 012 00). The inlet of each holder has been machined to a knife-edge at the tip of the inlet to produce a "thin-walled" sampler.[29] Two types of filter discs have been used in the holders: 0.2 µm pore size, track-etched, polycarbonate screen membranes with a plain surface using polyvinylpyrrolidone as a wetting agent (Poretics Corp., Livermore, Calif., catalog no. 10510) and 0.2 µm pore size, track-etched, polycarbonate screen membranes that are stained with irgalan black (Poretics Corp., catalog no. 10521).

Airflow through filters is controlled by regulating the vacuum applied to a plenum below each pair of holders through a fitting on the side of the sampler connected to a portable, flow-controlled vacuum pump (SKC Inc., Eighty Four, Pa., model 226-44XR). Six fittings are situated around the manifold, one for each pair of filter holders, to allow six sets of duplicate samples to be collected over time. One of the duplicates is always on the inner ring of filters, and the other is on the outer ring. With suitable connectors, vacuum can be applied to any combination of paired samplers. Flows are measured at each filter holder inlet with a filter in place using an electronic bubble meter (attached with flexible tubing) before and after sampling is conducted.

The airflows through the filter discs (0.3 to 1.1 L/min) are maintained approximately isokinetic with the flow through the cone. However, maintaining isokinetic sampling is not always necessary because sampling with the filters will often meet the criterion of "still" air sampling for particles with aerodynamic diameters <30 µm as established by Davies.[30,31] For still air sampling, the filter holder inlet diameter (0.87 cm) is large enough to cause less than a 10% sampling error according to the criterion of Agarwal and Liu for upward-facing nozzles.[32]

Flow through a pair of filters represents only a few percent of the total flow through the sampling device and thus limits effects on the flow field within the cone. The total flow includes the flow through the individual filters, 2 to 5 L/min flow to a particle collector or counter, respectively (as described below), and the bulk flow, i.e., the balance selected to maintain total flows of 60 to 120 L/min, which is pulled through the openings between the sample pairs (see Figure 2). The bulk flow is provided by a ring compressor (Fuji Electric Corp. of America, Lincoln Park, N.J., model VFC083P) calibrated with a dry gas meter.

The central cavity in the sampling ring leads to a vertical pipe with a 2.1 cm i.d. that is connected to the external particle collector or counter. This feature allows the determination of particle concentrations and size distributions within the horizontal plane of the filter holder inlets. Flows of 2 to 5 L/min through the central cavity correspond to velocities of 0.09 to 0.22 m/sec in the plane of the manifold. For the air velocities that have been maintained in this plane during the evaluation of the sampling device (0.06 to 0.25 m/sec), sampling through the central cavity is essentially either isokinetic or satisfies still air sampling requirements. In either case the sampling error is expected to be less than 10% for particles with aerodynamic diameters <30 µm in all tests, according to the criteria for still air sampling referred to in the previous paragraph or according to the anisokinetic losses predicted by Belyaev and Levin.[29]

Laboratory Evaluation of the Manifold Sampler

Wind Tunnel and Aerosol Generation System

To test the sampling device, a uniquely compact wind tunnel was constructed. Its development and evaluation have been reported in detail by Eddins et al.[33] The wind tunnel was constructed from a polyvinyl chloride pipe, 305 cm in length with an i.d. of 59 cm, corresponding to a cross-sectional area of 0.273 m^2. The inside of the pipe was coated with a paint containing graphite that rendered the internal surface electrically conductive. The axis of the pipe was oriented horizontally, and a high efficiency particulate air (HEPA) filter capped each end to purify the inlet air and to remove the generated test aerosol from the exhausted air. A centrifugal fan pulled air through the system so that the tunnel was under negative pressure. A speed control on the fan motor provided continuous adjustment of the velocity in the tunnel from 0.2 to 2 m/sec.

Test particles were injected through a jet pump operating at 34 L/min of airflow and oriented so that its axis coincided with the inlet end of the wind tunnel axis. A deflection plate (6-cm diameter disk) was located perpendicular to and 5 cm from the jet pump exhaust stream. Mixing of particles with the total gas entering the wind tunnel was accomplished by turbulence produced by this axial jet pump combined with two additional opposed jet pumps. These latter two jet pumps were located on the horizontal diameter of the wind tunnel with their jets directed toward the deflection plate at the axial jet pump where particles were injected. Airflow rate from the jet pumps was less than 2% of the total wind tunnel airflow.

Test aerosols for the evaluation of the sampling device were generated with the TSI Model 3400 fluidized bed aerosol generator (TSI, Inc., St. Paul, Minn.) and transported to the axial jet pump. Three commercially available materials were used: Zeeosphere® ceramic beads (type X-62, Zeelan Industries, Inc., St. Paul, Minn.) with a reported density of 2.25 g/cm^3, and polystyrene beads of two average sizes, 14.6 µm and 24.3 µm, both with a density of 1.15 g/cm^3 (Bangs Laboratories, Inc., Carmel, Ind.). The ceramic beads produced a aerosol with an mass median aerodynamic diameter (MMAD) of 11.9±0.5 µm and a geometric standard deviation (GSD) of 1.57±0.01. The polystyrene beads produced nearly monodisperse distributions with MMADs near expected values. Generation of the smaller polymeric beads yielded an MMAD of 16.2 µm with a GSD of 1.2, and the larger beads, an MMAD of 25.6 µm with a GSD of 1.1. Particle feed rates were established to provide concentrations ranging from about 0.9 to 5.6 mg/m^3 with the ceramic beads and 0.3 to 1.6 mg/m^3 with the polystyrene beads. Wind tunnel velocities ranged from 0.2 to 1.4 m/sec.

During particle sampling experiments, the manifold sampler was situated with its vertical axis on a vertical diameter of the wind tunnel and 40 cm from the HEPA filter at its exit. The horizontal plane of the inlet slit at the top of the sampler was positioned to coincide with the horizontal diameter of the wind tunnel. In this position the manifold sampler blocked 14% of the wind tunnel

cross section. This is less than the 15% maximum specified by the Environmental Protection Agency for testing of PM_{10} samplers.[34] Reference particulate samples of the gas stream were extracted through a sampling nozzle located on the wind tunnel axis 20 cm upstream of the manifold sampler axis and transported downward through a vertical probe for determinations of particle concentrations and size distribution.

Evaluations of the spatial uniformity and temporal stability of gas velocity and particle concentrations and size distribution were reported by Eddins et al.[33] For average wind tunnel velocities ranging from 0.2 to 1.6 m/sec, 20-point velocity traverses were performed. Observed average gas velocities at each point were typically within ±5% of the total traverse average. In the central region of the wind tunnel, where the manifold sampler inlet slit and the reference sample probe inlet were located during the sampling-efficiency experiments, deviations were always less than ±10%. Along the horizontal and vertical diameters this degree of uniformity existed for points within 25 and 13 cm of the center, respectively. Maximum within-point variations of instantaneous axial gas velocities ranged from ±14 to ±2% for average velocities of 0.2 to 1.6 cm/sec, respectively.

The degree of mixing, with the total wind tunnel flow, of gas injected through the axial jet pump was evaluated by injecting a tracer gas through this pump and performing concentration traverses. The results had between-point coefficients of variation (CVs) from 5 to 9% and within-point CVs from 4 to 6% for average wind tunnel velocities from 0.2 to 1.4 cm/sec, respectively.[33]

Figure 3 presents concentration as a function of particle size for reference samples of the ceramic beads obtained at six points along the vertical diameter for a wind tunnel velocity of 0.4 cm/sec. Each data point is the average of two, three, or four 5-min sampling runs. The total data set was obtained over a period of 5 hr. Particle concentration was nearly uniform over the entire particle size range except for the point near the top wall of the wind tunnel (9 in. above in Figure 3). The low concentrations of larger particles at this point were likely the result of gravitational sedimentation in the wind tunnel. Also, concentrations of larger particles at the other points exhibit a trend, although with much smaller differences, toward higher values at the lower points. In the central region of the wind tunnel, i.e., excluding the top and bottom points, variations of particle concentrations were less than ±10% for particle sizes less than 15 µm and less than ±20% over the entire size range. It is believed that along the horizontal diameter between-point variations of particle concentration were substantially less than those observed along the vertical diameter because of the absence in this plane of variation caused by gravitational sedimentation.

Inertial and sedimentation losses of these ceramic beads in a horizontal sampling probe prevented acquisition of particle-concentration data while traversing the horizontal diameter to verify the expected lower between-point variation. However, comparison of horizontal and vertical traverses obtained with somewhat smaller particles (MMAD of 5 µm and GSD of 1.8), for which probe losses were small, indicated much less variation (by factors of one-half and less) along the horizontal diameter than along the vertical diameter. These results also indicated that variations along the horizontal diameter were independent of average wind tunnel velocity while variations along the vertical diameter increased with decreasing average wind tunnel velocity.

Aerosol Measurement

In the primary laboratory experiments, particle concentrations were determined as a function of size with the TSI aerodynamic particle sizer (APS model no. 3310). The APS was alternately positioned under the vertical pipe of the manifold sampler exhaust and the vertical probe used for reference samples of the wind tunnel gas stream. At either location and under each set of test conditions, aerosol concentrations were determined with the APS over an averaging time of either 5 or 20 min; the alternate measurements were repeated at least three but usually four times.

In laboratory tests with the polystyrene beads, samples collected with the 13-mm filters of the manifold sampler were examined by bright field microscopy as follows: Each filter disc was mounted on a slide in a methylene blue solution to increase contrast between the beads and the filter. The filter was then observed and particles counted at a magnification of 400× with a Nikon OPTIPHOT-POL microscope equipped with an image analysis system—a Newvicon camera (mti™ 70 Series, Dage-MTI Inc., Michigan City, Ind.) with Image-Pro Plus software (Media Cybernetics™, Silver Spring, Md.).

Samples taken with polycarbonate filter discs in the field were also examined by bright field microscopy after being mounted on Cyto•Clear™ glass slides (Poretics Corp., catalog no. 99030) in 4 µL of glycerin jelly, a mixture of 7.5 g of

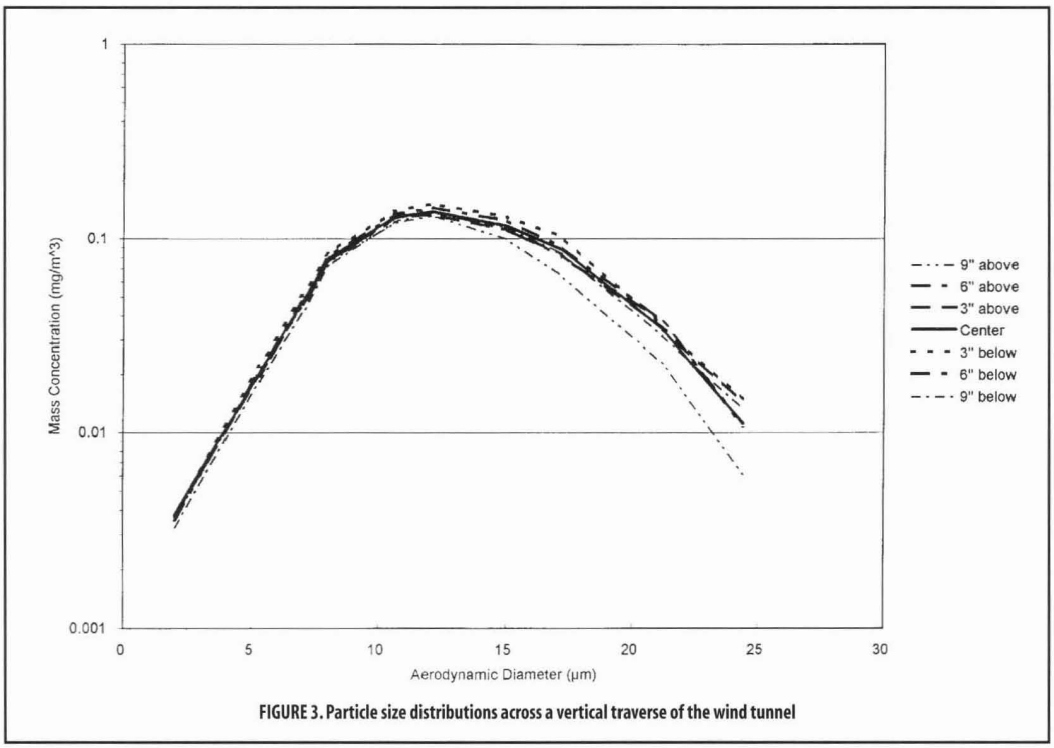

FIGURE 3. Particle size distributions across a vertical traverse of the wind tunnel

gelatin in 75 mL sterile distilled water and 100 mL of glycerol. Fungal spores were counted in the field samples at 1000× magnification using the image analysis system.

Design and Evaluation of Sampling Efficiency of Probes for the APS 3310

Test particles were extracted from the aerosol stream and transported to the APS sensor through a fabricated probe for the purpose of measuring concentrations in the wind tunnel. A probe design was selected to optimize aspiration efficiency and to minimize wall deposition in transit.[35] The sampling probe was comprised of a thin-wall nozzle, 2.1 to 2.7 cm in length, placed at an angle of 45° to the airstream and attached through a 45° elbow to a straight, vertical, stainless steel pipe 55 cm in length and 1.6 cm i.d. This transport pipe was connected to the vertical inlet (1.9 cm i.d.) of the APS 3310. Because the APS was operated at one volume flow rate (i.e., 5 L/min), nozzles with diameters ranging from 0.77 to 2.30 cm were used to accommodate the various wind tunnel test velocities. Nozzle velocities were maintained somewhat lower than the velocity of the airstream (i.e., subisokinetic) to oversample and thus compensate for the expected loss of large particles in the probe.

Probe sampling efficiencies were determined at the various wind tunnel velocities by gravimetric measurement of aerosolized type X-62 Zeeosphere ceramic beads collected on 25-mm glass fiber filters (Gelman Sciences, Ann Arbor, Mich., catalog no. 61630) mounted in graphite-filled polystyrene cassettes with extended cowls (Environmental Express, Mt. Pleasant, S.C.). For each determination, aerosol was sampled simultaneously inside the wind tunnel and through the sampling probe fitted with the appropriate nozzle. The cassette inside the wind tunnel was placed immediately adjacent to the nozzle and sampled isokinetically; whereas the other cassette was attached to the exit of the probe outside the wind tunnel and sampled at 5 L/min. The corresponding ratio of the mass concentration determined with the filter attached to the probe, to the mass concentration determined with the filter inside the wind tunnel was entered into the column labeled "Observed Efficiency" in Table I.

TABLE I. APS Probe Sampling Efficiency[A]

Wind Velocity (m/sec)	Sampling Velocity (m/sec)	Sampling Time (min)	n[B]	Observed Efficiency[C] (%)	Predicted Efficiency[D] (%)
0.2	0.2	90	3	92.3 (2.0)	95.1
0.4	0.2	90	3	93.1 (5.9)	93.6
0.6	0.2	150	3	96.2 (3.0)	98.2
1.0	0.6	180	3	90.2 (5.1)	89.7
1.4	1.0	180	3	83.9 (6.5)	85.5

[A]These tests were carried out with type X-62 Zeeosphere ceramic beads (MMAD=11.9 µm, GSD=1.57).
[B]Number of test runs
[C]Observed probe sampling efficiencies calculated as specified in the text. Data in parentheses are the CVs of observed efficiencies expressed as a percent.
[D]Probe sampling efficiencies predicted using equations in References 35–37 and specifications in text

These observed probe sampling efficiencies were compared to predicted efficiencies based on the model for nozzles developed by Hangal and Willeke[35,36] and on the model of inertial deposition losses in a bend developed by Crane and Evans.[35,37] Prediction of total sampling efficiency for the aerosol samples requires particle size distribution data. These data were obtained immediately before and after each filter experiment by APS measurements, sampling through the probe to determine the apparent concentration and size distribution. As measured with the APS, the MMAD changed by no more than 0.3 µm and the GSD by no more than 0.02 during any of the filter runs.

To compute the predicted overall probe sampling efficiency for a particular wind velocity, the apparent mass concentration corresponding to one particle size bin of the APS measurement was corrected for the predicted probe losses to yield an estimate of the mass concentration in the wind tunnel. This process was repeated for each particle size bin of the APS. The corrected concentrations were summed over all of the APS bins, i.e., summed over the particle size distribution. The overall concentration as determined directly with the APS was then divided by the sum of corrected concentrations to yield the overall theoretical efficiency of the sampling probe for the polydisperse aerosol. This value was entered into the column labeled "Predicted Efficiency" in Table I.

The predicted results were in good agreement with the observed efficiencies; a paired two-tailed t-test at the 0.05 significance level revealed no statistical difference between experimental and theoretical efficiencies. The average observed total bias was 1.5%. Another series of tests was carried out at a sampling velocity of 0.4 m/sec and at a wind velocity of 0.4 m/sec using the 25.6-µm polystyrene beads. These tests yielded an average probe efficiency of 82.3% (CV=2.1%, n=2) compared with 82.1% predicted with the theoretical model. Because the accuracy of the theoretical model was good in all of these tests, the model was used to correct the APS results within each individual particle size bin in sampling efficiency studies of the manifold sampler.

Laboratory Determination of Sampling Efficiencies of the Manifold Sampler

Tests with ceramic spheres were conducted over particle sizes ranging in aerodynamic diameter from 2.0 to 24.4 µm and at air volume flow rates of 30, 60, 90, and 120 L/min through the inlet of the manifold sampler, corresponding to sampling velocities of 0.2, 0.4, 0.6, and 0.8 m/sec at an inlet slit height of 0.3 cm. Wind tunnel velocities were adjusted to 0.2, 0.4, 0.6, 1.0, and 1.4 m/sec, covering the range encountered in most indoor environments.[38]

Of the 59 particle size bins of the APS, those representing the following particle sizes were chosen for sampling efficiency calculations: 2.0, 5.3, 7.0, 8.0, 10.6, 12.1, 15.0, 17.2, 21.2, and 24.4 µm. The sampling efficiency for a given particle size was determined by dividing the average concentration recorded by the APS when connected to the manifold sampler by the average concentration recorded when connected to the reference probe in the wind tunnel. Although variations in aerosol concentration over the time spanning a set of replicate tests were small, the particle size distribution was even more stable.[33] Therefore, to maximize precision, concentrations observed within each of the selected particle size bins were normalized to concentrations observed for particles less than 2 µm, based on the assumption that sampling efficiencies for these small particles would be near unity. A similar normalization was performed by Marple and McCormack in the evaluation of a personal sampling impactor.[39]

For 5-min sampling times, ceramic spheres were used to evaluate sampling efficiencies for aerodynamic sizes up to 21.2 µm. Above this diameter, measurement precision was low, i.e., CVs were greater than 20%. Consequently, the monodisperse polystyrene beads of 25.6 µm aerodynamic diameter were used for determining sampling efficiencies for a particle size above 21.2

μm. With these polystyrene beads and for 5-min sampling times, a CV of 15% was observed for replicate measurements. For a portion of the tests at 0.8 m/sec sampling velocity, 20-min sampling times were used to increase counts, thus allowing more precise measurements (i.e., CVs less than 20%) with ceramic spheres up to 24.4 μm in diameter. For a more thorough discussion of sampling precision, see Results and Discussion and Table II.

TABLE II. Precision of Replicate APS Measurements of Particle Concentration

Aerodynamic Diameter, μm	CV[A] (%) for 5-min Sampling Time	CV[A] (%) for 20-min Sampling Time
2.0	2.0	1.4
5.3	2.8	1.9
7.0	2.9	1.7
8.0	3.2	1.9
10.6	4.3	3.8
12.1	4.5	4.4
15.0	6.7	5.1
17.2	8.5	6.7
21.2	16.0	10.6
24.4	44.2	18.4
25.6	15.0[B]	—

[A]Coefficient of variation pooled over all wind velocities studied
[B]Tests with monodisperse polystyrene beads

Laboratory Evaluation of the Accuracy and Precision of the Manifold Sampler

Two test procedures were employed. In the first the effect of different combinations of filter pairs on the particle size distribution and concentration in the plane of the manifold was determined. Type X-62 Zeeosphere polydisperse ceramic beads were aerosolized in the wind tunnel and pulled into the manifold sampler for these tests. With the APS monitoring concentrations and size distribution of the polydisperse aerosol through the central cavity of the manifold, aerosol was sampled concurrently through one or more pairs of filters of the manifold at an average rate of 0.38 L/min through each filter. The total volume flow rate through the sampler was 60 L/min, corresponding to an inlet sampling velocity of 0.4 m/sec.

In the second group of tests, the bias and precision of count concentrations were determined by microscopic analysis of filter samples using the APS output as a reference. The APS monitored the concentration of monodisperse polystyrene beads entering the manifold sampler from the wind tunnel while filter samples were being taken simultaneously with all filter pairs. As in the tests described in the previous paragraph, an average sampling rate of 0.38 L/min was maintained through the filters, and a total volume flow rate of 60 L/min was maintained through the manifold sampler.

Field Evaluation of the Manifold Sampler

Field tests were conducted primarily to verify that precise, sequential measurements of actual bioaerosols could be performed in realistic settings. Three field tests were conducted; one in a cotton dust environment and two on a dairy farm.

Cotton Dust Environment

The first field test was carried out in a simulated cotton carding room, as a courtesy of the U.S. Department of Agriculture Laboratory at Clemson University. The manifold sampler was operated at a total volume flow rate of 45 L/min, corresponding to an inlet slit velocity of 0.3 m/sec; volume flow rate through each 13-mm polycarbonate filter averaged 0.35 L/min. Air was sampled about 1 hr sequentially through each of five filter pairs for a total sampling time of about 5 hr. No sampling was conducted through the sixth pair to provide filter blanks. Air was also sampled at a rate of 2 L/min concurrently through the central cavity of the manifold sampler and outside with two 25-mm, 0.2 μm pore size, track-etched polycarbonate filters mounted in open-faced graphite-filled polystyrene filter holders. The mass of cotton dust collected on each of these filters was determined gravimetrically and used to determine concentrations. These values were compared with reference particulate mass concentrations of the carding room determined by sampling with vertical elutriators. Fungal spores collected with the manifold sampler were counted to determine the precision of replicate samples and to determine a concentration-time profile.

In the cotton carding room tests, a personal volumetric air sampler (Burkard Manufacturing Co. Ltd, Rickmansworth, England, U.K.) was employed to sample bioaerosols near the inlet of the manifold sampler. The Burkard sampler impacted aerosol through a 2-mm slit onto a glass slide coated with a thin layer of petrolcum jelly at an air volume sampling rate of 10 L/min. Samples were taken with the Burkard sampler for 1 to 5 min intervals during sampling with the manifold sampler.

Dairy Farm Environment

The second and third field tests were conducted in a three-sided shed at a dairy farm located in rural Alabama during the mixing of cattle feed. In one test the total air volume flow rate through the manifold sampler was about 60 L/min, corresponding to an inlet slit velocity of 0.4 m/sec. Air movement around the sampler was primarily the result of the convective stirring of the air by the front end loader used to add different components of the feed to a mixing hopper. The wind velocity was <0.6 m/sec throughout the test and was not consistently from one direction. The air was sampled simultaneously through five filter pairs with the sampling manifold for 25 min at an average flow rate of 0.50 L/min (CV=6.9%). Air was not sampled through the sixth pair; these two filters served as blanks. Microscopic spore counts on the individual 13-mm filters were compared to assess sampling precision with the manifold sampler.

The major objective of the other field test at the dairy farm was to compare microscopic spore count concentrations determined sequentially to corresponding count concentrations found by sampling through the central cavity. At a volume flow rate of 60 L/min, corresponding to an inlet slit velocity of 0.4 m/sec, air was sampled sequentially for 10 to 12 min through four pairs of 13-mm polycarbonate membrane filters at an average flow rate of 0.5 L/min. The other two pairs served as blanks. Concurrent with the sampling through each filter pair in the manifold, air was sampled through the central cavity with a 25-mm polycarbonate filter in a graphite-filled cassette. The sampling rate for the cassette was 2.2 L/min. As in the first test, the wind velocity was below 0.6 m/sec throughout the test and was from varying directions.

RESULTS AND DISCUSSION

Sampling Efficiencies of the Manifold Sampler

The results of the sampling efficiency tests of the manifold sampler in the wind tunnel define the size separation characteristics of the

sampler as a function of wind speed. The observed sampling efficiencies are shown in Figures 4 to 7. Sampling efficiencies generally decreased with increasing wind velocity, decreased as particle size increased, and increased as sampling flow rates increased. The observation that sampling efficiency decreased as wind velocity increased was qualitatively consistent with the theoretical model by Hangal and Willeke describing nozzle efficiencies.[36] In a directed flow stream such as that in the wind tunnel, a portion of the air entered the slit of the manifold sampler in the same direction as the gas stream and other portions entered at different directions. These varying sample flow directions corresponded to isoaxial and anisoaxial sampling. According to the model, as the wind velocity increased, the greater sampling efficiencies expected for particles greater than about 5 µm by isoaxial sampling would be more than offset by the decreased efficiencies expected for anisoaxial sampling. A similar trend was noted by McKenzie et al.[40] for a personal aerosol impactor with a segmented circular slit inlet.

The precision of APS measurements was a function of the sampling time and the number concentration for each particle size. The CV pooled over all wind tunnel velocities for each of the particle sizes of interest and at sampling times of 5 and 20 min are presented in Table II.

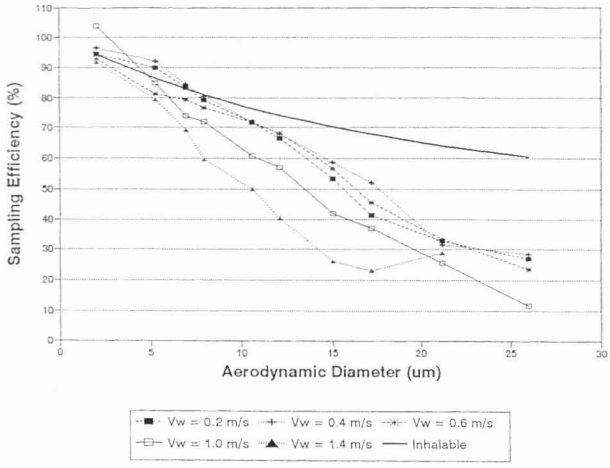

FIGURE 4. Sampling efficiency of the manifold sampler; sampling velocity at 0.2 m/sec

Although the sampling efficiency did change with wind speed, the magnitude of change appeared to be comparable to or less than that observed with the IOM area inhalable sampler.[26] However, comparison of the performance of the manifold and IOM samplers was complicated because the devices were not tested over the same range of wind velocities. Nevertheless with the IOM sampler, a three-fold change in velocity (from 1 to 3 m/sec) resulted in about 35% change in aspiration efficiency for particles with aerodynamic equivalent diameters near 15 µm and about 20% change for diameters near 20 µm. As shown for the manifold sampler in Figures 4–7, a three-and-half-fold change in wind speed (from 0.4 to 1.4 m/sec) demonstrated the following changes in sampling efficiency for 15-µm particles at the designated sampling velocities: 30% at 0.2 m/sec, 25% at 0.4 m/sec, 25% at 0.6 m/sec, and 15% at 0.8 m/sec; and for 20-µm particles, 10% at 0.2 m/sec, 20% for 0.4 m/sec, 20% at 0.6 m/sec, and 10% at 0.8 m/sec. The manifold sampler demonstrated much less change with wind velocity than did the segmented circular slit impactor developed by McKenzie et al.,[40] for which a change in wind velocity from

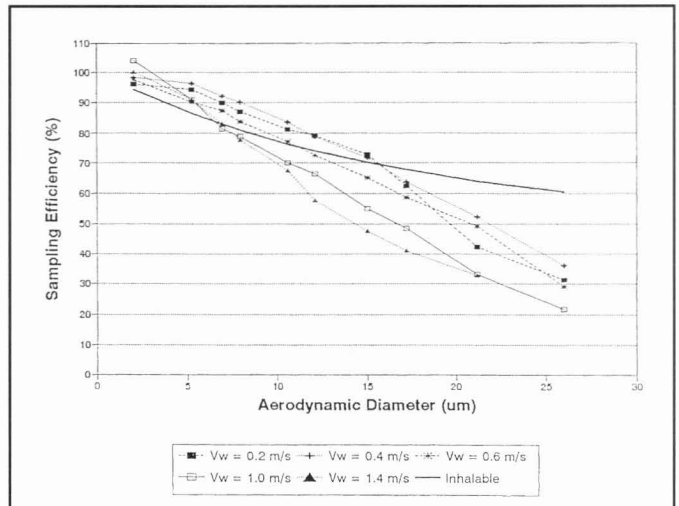

FIGURE 5. Sampling efficiency of the manifold sampler; sampling velocity at 0.4 m/sec

0.5 to 1.5 m/sec produced a 50 to 70% change in sampling efficiencies for 15-µm particles. A significant portion of the variability observed with the manifold sampler over the range of wind velocities for particle diameters >20 µm may have been caused by measurement error; see Table II.

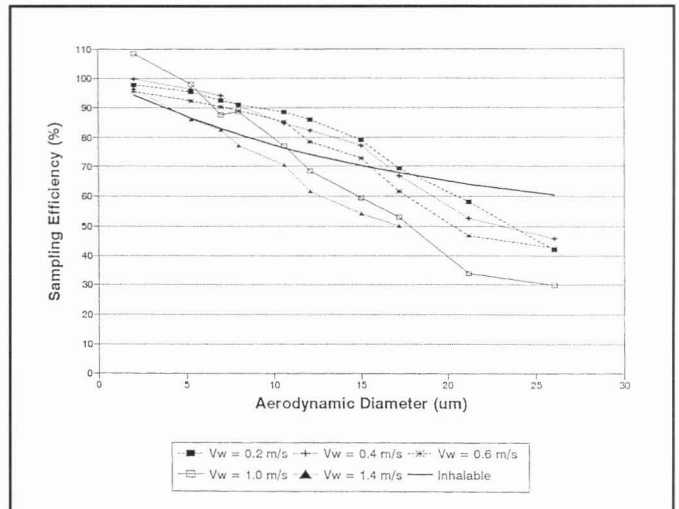

FIGURE 6. Sampling efficiency of the manifold sampler; sampling velocity at 0.6 m/sec

Comparison of Sampling Efficiencies of the Manifold Sampler with the ACGIH Inhalability Curve

For sampling velocities ≥0.4 m/sec, sampling efficiencies were higher than the ACGIH inhalability curve for relatively small particles and lower than the curve for relatively large particles. Sampling velocities of 0.6 and 0.8 m/sec demonstrated efficiencies that matched the ACGIH inhalability curve more closely than did results at the lower sampling velocities of 0.2 and 0.4 m/sec. For wind velocities ≤0.6 m/sec (≤~120 ft/min), which are seldom exceeded in indoor environments,[41] a sampling velocity of 0.6 m/sec yielded efficiencies within 10% of the inhalable curve up to particle diameters of about 18 µm and efficiencies within 18% for diameters up to 26 µm. At wind velocities ≥0.6 m/sec, a sampling velocity of 0.8 m/sec yielded efficiencies that were within 15% of the inhalable curve up to diameters of about 22 µm and

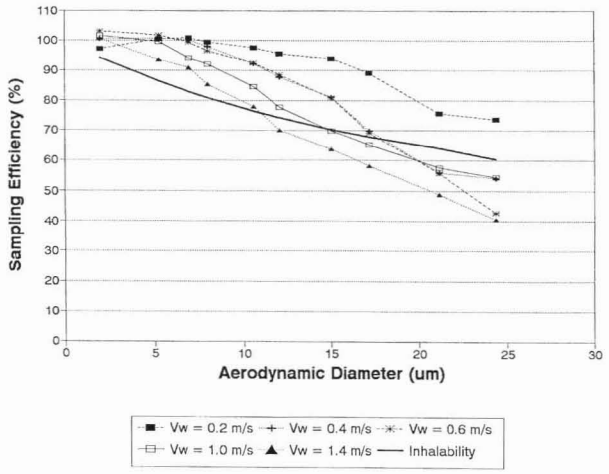

FIGURE 7. Sampling efficiency of the manifold sampler; sampling velocity at 0.8 m/sec

within 20% for diameters up to 25 µm. Most airborne bacteria and fungal spores are expected to have aerodynamic equivalent diameters <30 µm.[41] Thus, the manifold sampler operated at a sampling velocity of 0.6 m/sec for most indoor environments or at 0.8 m/sec for "breezy" indoor environments (where wind velocities may be >0.6 m/sec) would be expected to collect the portion of bioaerosols that reasonably corresponds to the inhalable fraction.

Performance of the Manifold of the Manifold Sampler

Additional tests in the wind tunnel revealed that sampling with any combination of filters in the manifold of the manifold sampler did not significantly change the concentration or particle size distribution inside the sampler. Figure 8 presents APS measurement results with the polydisperse ceramic beads while sampling was concurrently conducted with the sampling manifold. In 45 consecutive 5-min tests of randomly selected combinations of filter pairs, the average observed levels (and CVs) were as follows: Count concentration = 3.91 x 10^7 counts/m^3 (5.7%), mass concentration = 2.07 mg/m^3 (6.8%), CMAD = 1.5 µm (1.6%), MMAD = 11.5 µm (1.4%), and GSD = 1.6 (0.4%).

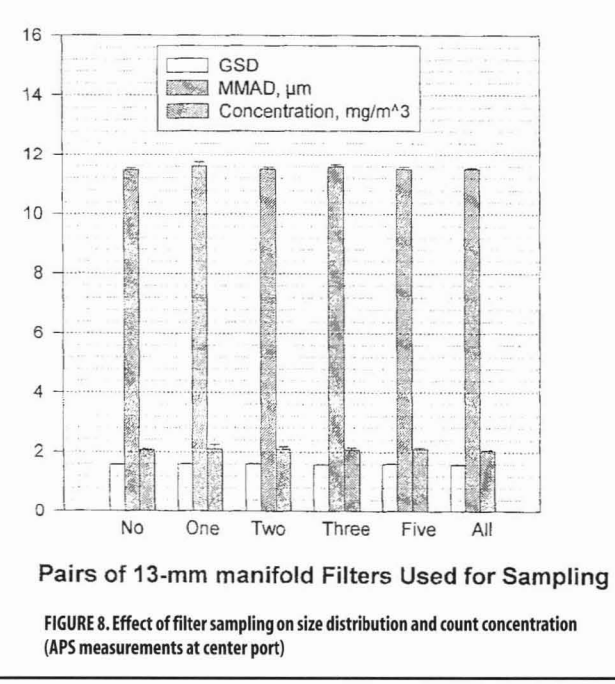

FIGURE 8. Effect of filter sampling on size distribution and count concentration (APS measurements at center port)

Additional tests with both 15.6 µm and 25.6 µm monodisperse polystyrene beads indicated reasonable agreement among the count concentrations determined by microscopic examination of the filter pairs. The results are presented in Table III. Two-sample t-tests revealed no statistical difference between concentrations of beads determined with the inside ring of filter holders and concentrations determined with the outside ring. In the test with 15.6 µm beads, precision was considered acceptable with an overall CV of 9.0%. With the 25.6 µm beads, the CV of count concentrations for samples in the outside sampling ring was far higher than expected and was attributed to the low count obtained with one of the filters. The value of 0.84 x 10^4 was classified as a statistical outlier by Dixon's criterion (p=0.04).[42] A much more respectable CV of 4.2% was obtained when the outlier was not included. All CVs were higher than expected from the error associated with the counting analysis step alone, i.e., the Poisson CV,[43] which is defined as the reciprocal of the square root of the count. For counts near 850 beads, the average count of 15.6 µm beads, the CV attributed to counting error would have been 4.3%, and 2.2% for counts near 1936, the average count of the 25.6 µm beads. One factor that may have increased the variability and may explain the outlier is the possibility that these relatively large polystyrene beads may have fallen or rolled off of the polycarbonate filter discs. The concentration measured through the central cavity as determined by the APS was 13% lower than the average of results determined with the filters for 15.6-µm beads and was 8% higher for 25.6 µm beads, neglecting the outlier. The counts of beads found on the duplicate set of blanks were only about 4% of the average count on filters through which air had been sampled. The blank counts were, therefore, considered negligible when compared to the challenge concentrations.

TABLE III. Distribution of Monodisperse Polystyrene Beads Inside the Sampler

Bead Diameter (µm)	Inside Ring (Count/m^3)×10^{-4}	Outside Ring (Count/m^3)×10^{-4}	Center by APS (Count/m^3)×10^{-4}
15.6[A]	16.4	17.6	
	16.5	14.6	
	18.6	18.0	
	19.2	15.2	
	17.1	18.5	
	Avg =17.6 (CV = 7.2%)	Avg =16.8 (CV = 10.5%)	14.9
25.6[B]	1.47	1.66	
	1.45	1.53	
	1.48	0.84[C]	
	1.29	1.57	
	Avg =1.42 (CV = 6.3%)	Avg =1.40[D] (CV = 26.9%)[E]	1.62

[A]On the average 250 fields containing 850 beads were counted on each filter. During the test the wind velocity was 1 m/sec and the sampling velocity was 0.4 m/sec.
[B]All beads caught on each filter were counted; the average count was 1930 beads. Both wind and sampling velocities were 0.4 m/sec.
[C]An outlier by Dixon's criterion. See text for reference.
[D]The average count concentration excluding the outlier was 1.59×10^4/m^3.
[E]The CV excluding the outlier was 4.2%.

Field Tests

In all field tests the precision of filter determinations of fungal spores with the manifold sampler was found to be near the Poisson CV, and the average count concentrations of replicate pairs were in close agreement with concentrations determined through the central cavity port of the manifold sampler.

In the first field test the manifold sampler was operated at a volume flow rate of 45 L/min, corresponding to a velocity of 0.3 m/sec at the inlet slit, and therefore was expected to perform with sampling efficiencies intermediate between those found in Figures 4 and 5. Sampling efficiency was expected to match the inhalable curve up to about 15 to 16 µm. A PCAM light scattering photometer (PPM, Inc., Chattanooga, Tenn.) mounted in the room determined the cotton dust concentrations to be around 1 mg/m^3 with a mass median diameter of the cotton dust to be 6.7 µm and a GSD of 2.4. Thus, 95% of the cotton dust particles were expected to have an aerodynamic equivalent diameter of <16 µm. Cotton fibers were also present; the nominal equivalent aerodynamic diameter of individual cotton fibers has been reported to be in the range of 30 to 45 µm.[44]

The count concentrations of fungal spores determined with the duplicate sets of manifold filters were found to be precise with a pooled CV of 10.1%. The overall precision was estimated from the paired differences of the duplicate manifold samples.[45] On the average about 300 fields were randomly selected on each of the filters to allow the counting of at least 100 spores. Two individuals counted the spores on each filter. On the average, one counter obtained results that were 6% lower than the other. Spore concentrations averaged 1.28 x 10^5/m^3 over the 5-hr sampling period.

Perhaps the most important finding of this field study is shown in Figure 9. The 1-hr averages of fungal spore concentrations exhibited a concentration-time profile that was similar to that for data obtained with vertical elutriators (VEs). A Burkard spore trap placed near the manifold sampler gave unsatisfactory results because cotton fibers impacted onto the collection slides made microscopic spore counting unreliable. This prevented a meaningful reference for fungal spore count concentrations. As expected, the filter samples collected with the manifold sampler did not suffer from this problem because the sampler has low sampling efficiencies for large particles such as cotton fibers. The fibers were trapped in the inlet and inside the cone, but none were observed on the filters.

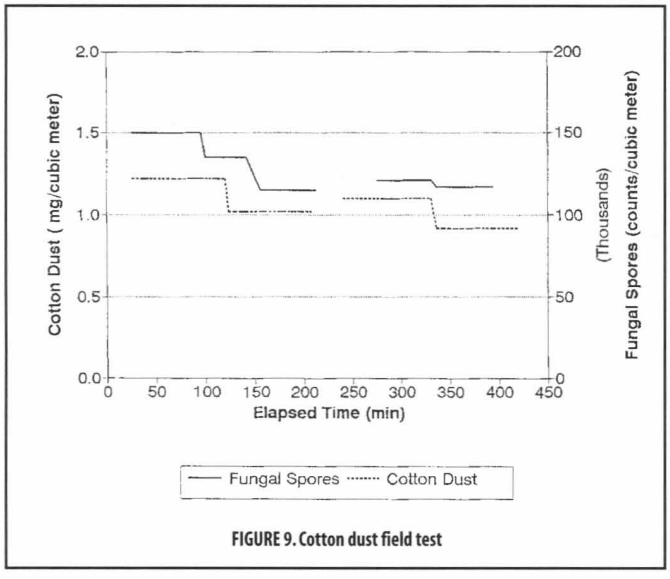

FIGURE 9. Cotton dust field test

Although the accuracy of fungal spore concentrations could not be assessed in this field study, cotton dust concentrations found with the manifold sampler were found to compare favorably to VE sampling results and PCAM aerosol monitor results. For example, the cotton dust level found by filter sampling from the central cavity inside the manifold sampler during the last hour of sampling was 0.76 mg/m^3. A cotton dust VE sample taken near the sampler and overlapped in time with the taking of the filter sample yielded a concentration of 0.77 mg/m^3, and the PCAM aerosol monitor readings averaged 0.82 mg/m^3 over the same hour.

The tests conducted at the dairy farm confirmed that spore concentrations could be determined with good precision with the duplicate pairs of the sampling manifold. In the first test in which sampling was conducted through five sets of filter pairs simultaneously, two-way analysis of variance revealed no significant effect of position within a duplicate pair (p=0.26) and no significant effect of position among the different pairs of samplers (p=0.95). As shown in Table IV, the pooled CV for within-pair variability was 6.9%, whereas the CV among pairs was 8.6%. An average of 448 microscopic fields was examined on each filter to obtain fungal spore counts near 100. The observed CVs were close to the Poisson CV of 10% indicating that the observed variability could be attributed to the precision of the microscopic count. Two sets of filters in the manifold were treated as blanks. The spore counts observed with these samples were small and considered negligible in comparison with the other samples. Center cavity port sampling was precluded by the failure of an air sampling pump in the field.

TABLE IV. Distribution of Fungal Spores Inside the Sampler[A]

Sample Pair No.	Inside Ring (Count/m^3)×10^{-4}	Outside Ring (Count/m^3)×10^{-4}
1	7.47	6.41
2	7.42	7.85
3	6.45	6.51
4	6.18	7.12
5	6.80	6.55
	Avg = 6.86 (CV = 8.4%)	Avg = 6.89 (CV = 8.8%)

[A]The pooled CV for filter pairs was 6.9%. Each pair was a filter in the outside ring and the corresponding filter in the inside ring on the same radius of the manifold.

In the second test at the dairy farm, samples taken sequentially with the filter pairs were compared with samples taken through the central cavity. The results are presented in Table V. The fungal spore count concentrations were higher than observed in the first test at the farm; consequently, fewer microscopic fields were examined to obtain spore counts near 100. The pooled within pair CV

TABLE V. Fungal Spore Count Concentrations: Manifold Versus Central Port

Run No.	Inside Ring (Count/m^3) ×10^{-5}	Outside Ring (Count/m^3) ×10^{-5}	Central Port (Count/m^3)×10^{-5}
1	2.91	3.24	3.74
2	10.5	9.62	9.53
3	4.77	5.91	5.50
4	5.07	4.44	4.85

was 10.1%, slightly higher than observed previously. The average of each replicate determination was compared to the count concentration determined from the central cavity sample using a two-tailed, paired t-test. No statistically significant bias was found (p=0.72). Blanks were again low and negligible.

SUMMARY AND CONCLUSIONS

The evaluation of the manifold bioaerosol sampler focused on size dependence of sampling efficiency and its ability to collect meaningful replicate and sequential filter samples. Tests were conducted with laboratory-generated aerosols in the size range of fungal spores and bacteria. Three field tests were conducted to assess the performance of the manifold sampler in the determination of fungal spores.

Test results indicate that the sampler is suitable for bioaerosol sampling and offers several advantages that are not features of other bioaerosol samplers. The device sampled with efficiencies that were similar to those represented by the ACGIH inhalability curve up to an aerodynamic equivalent diameter near 30 µm at a volume flow rate of 90 L/min for wind velocities of 0.2 to 0.6 m/sec, and at a flow rate of 120 L/min for wind velocities ≥0.4 m/sec. Laboratory and field evaluations demonstrated its utility in collecting sequential and replicate samples. The sampling manifold is capable of collecting up to six duplicate samples sequentially. A random combination of 2 to 12 replicate samples did not affect the particle size distribution or aerosol concentration in the plane of the sampling manifold. Sampling flow rates through each filter holder in the manifold were typically maintained at 0.3 to 0.5 L/min; however, flow rates up to about 1.1 L/min could be accommodated. A rate of 1.1 L/min would be isokinetic for a total rate of 120 L/min but should also provide accurate still air sampling at total rates <120 L/min. A center port allowed sampling at 5 L/min for the use of the APS 3310 aerodynamic particle sizer or at 2 L/min for the use of 25-mm filters. Field tests indicated that the precision of filter samples obtained with the manifold sampler was near that predicted by the Poisson CV for microscopic counting of the filter catch. The CV did not appear to be a function of concentration; CVs near 10% were obtained for spore count concentrations ranging from about 10^4 to 10^6 counts/m^3 when about 100 spores were counted on each filter. Biases between spore counts observed for the manifold filters and counts observed with the central cavity port were reasonably low, averaging –4.3%.

IMPLICATIONS

The manifold sampler is unique in its ability to allow replicate and sequential particle samples to be taken in the inhalable size range for not only bioaerosols but also for many other occupational or environmental dusts. The device is particularly suited for filter sampling of environments contaminated with biogenic dusts. Viable bioaerosol impactor sampling in such environments cannot be used without considerable difficulty because the agar plates are easily overloaded.[46] A further advantage of the manifold sampler is the capability to filter the bulk airflow downstream of the manifold, thereby providing a high volume sample for analysis.

Using a sensitive analysis method, e.g., bright field or epifluorescence microscopy, one can likely use the manifold sampler to determine the relatively low levels of bioaerosols found in so-called "uncontaminated" environments. Assuming a Poisson distribution of bioaerosol counts, the limit of detection (LOD) of the method is estimated to be 3, i.e., three times the standard deviation of a count of 1. Blank polycarbonate filters have been consistently found to be free of bacteria and fungal spores. For low particle densities, the entire filter can be examined within a reasonable time by manual or automated rapid scanning. For a 1-hr air sample taken with a 13-mm filter at 1 L/min, an LOD corresponding to about 50 particles/m^3 is estimated.

The manifold sampler allows filter sampling amenable to microscopic examination for the determination of total bioaerosol counts. As pointed out by Eduard et al.,[46] filtration using membrane filters offers several advantages over other methods of sampling. The filter catch may be assessed in several different and complementary ways, including bright field and epifluorescence microscopy or scanning electron microscopy. In the present study the 0.2 µm pore-size polycarbonate membrane filters have been found suitable for bright-field microscopic examination with digital image analysis when the pores are cleared with Cyto•Clear slides. In related laboratory studies epifluorescence microscopy has been found suitable for the enumeration of bacteria on blackened polycarbonate filters.[47] The 0.2 µm pore size ensures the quantitative sampling of biogenic particles, including bacteria and fungal spores.[48] Furthermore, the high filtering efficiency ensures that the particle sizing features of the manifold sampler will not be compromised.

The filter catch is also suitable for the chemical determination of biogenic substances, including endotoxins, (1→3)-β-D-glucans, and mycotoxins such as aflatoxins. The ability to take replicates with the manifold sampler allows the collection of identical samples that may be subjected to totally different analysis techniques for the determination of several contaminants within the same air sample.

Further field and laboratory tests are underway to apply the manifold sampler to the determination of bioaerosols, including bacteria, in several different types of heavily contaminated environments. In these tests the microscopic counts determined with the use of the manifold sampler are being compared with counts determined by complimentary techniques, including viable sampling. Additional tests are underway at the dairy farm, and other tests are planned for a contaminated building and a poultry confinement building.

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