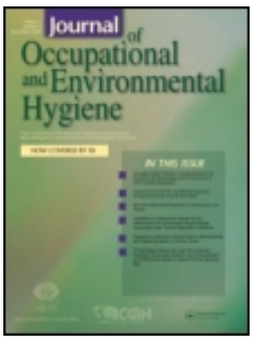

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Chamber Evaluation of a Personal, Bioaerosol Cyclone Sampler

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*A personal cyclone sampler (cyclone) was operated in a 0.9-m^3 chamber, side by side with a 25-mm filter sampler (filter) and either a slit impactor (Air-O-Cell) or a single-stage, multiple-hole, agar impactor (N6). Aerosols of two fungal spores were collected for 5 min to 5 hr—*Aspergillus versicolor*: 10, 20, 40, 80, 160, and 320 min; concentration: 10^2–10^5 spore m^{-3};* Scopulariopsis brevicaulis*: 5, 10, 15, 20, 25, and 30-min; concentration: 10^3–10^5 spore m^{-3} (six replicates for each sampling time). For each fungus, air concentrations were determined by a 15-channel optical particle counter (particle m^{-3}; N = 36), microscopy (spore m^{-3}; cyclone and filter, N = 36; Air-O-Cell, N = 18), culture (colony forming unit m^{-3}; cyclone and filter, N = 36; N6, N = 18), and polymerase chain reaction (cell equivalent m^{-3}; cyclone and filter, N = 36). Samplers were significantly correlated with each other as were the three analyses (correlation coefficients = 0.79–1.00 and 0.87–0.98, respectively). Ratios were calculated for simultaneous measurements with the cyclone and comparison samplers and for paired colony:spore, colony:cell equivalent, and cell equivalent:spore measurements for the cyclone and filter samples. The cyclone equaled or underestimated the other samplers for both fungi and all analyses (mean ratio: 0.75–1.04).* A. versicolor *colony and cell equivalent measurements exceeded spore measurements although microscopy should detect all spores not just culturable ones, perhaps due to difficulty observing the smaller spores or detection of DNA in cell fragments in addition to intact spores. Plots of the ratios of paired measurements against their averages identified biases between samplers and analyses. For example, ratios were correlated with spore concentration, and there was greater uncertainty at lower concentrations. These chamber tests have shown that the cyclone is suitable for collection of airborne fungal spores over a wide concentration range and time period and for analysis by microscopy, culture, and polymerase chain reaction.*

Keywords airborne fungal spores, chamber study, cyclone sampler, method comparison, sampler performance

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

INTRODUCTION

Conventional methods for the measurement and identification of airborne fungal spores have been criticized for their reliance on time-consuming and labor-intensive microscope and culture assays, which limit routine collection of air samples for the study of plant, animal, and human diseases.[1,2] Furthermore, methods based on counting spores and colony-forming units (CFUs) can be imprecise when cell density is low, and the variable accuracy of human analysts can weaken comparisons across studies.[3]

Many fungal spores can be identified only to the genus or group level by microscopy, and in culture, some fungi either cannot reproduce, grow but do not produce spores necessary for identification, or reproduce slowly and can be overgrown by other fungi or bacteria.[4] Therefore, over the past decade, molecular techniques have been recognized as alternative and supplementary methods for the detection of airborne fungi.[5–8] Collection of spores directly into a vessel suitable for polymerase chain reaction (PCR), immunoassay, or chemical analysis also has been considered an attractive option to conventional spore sampling onto glass slides, agar, or filters.[2,9]

The performance of a personal aerosol sampler based on cyclone principles, which uses a 1.5-mL microcentrifuge tube (MCT) as a particle receptacle (Figure 1), has been evaluated for collection of fluorescently tagged polystyrene latex (PSL)

FIGURE 1. Personal cyclone sampler with microcentrifuge tube (MCT) (18-mm coin shown to indicate scale)

particles and polymer microspheres[10] as well as ambient fungal spores.[11] In addition to being easy to use, simple to fabricate, and inexpensive, long-term samples are possible with the cyclone, there is no sample transfer loss, particle recovery is high, and multiple analyses can be performed on each sample. A two-stage version of the cyclone also has been characterized for collection of PSL particles and fungal spores.[12]

The current evaluation of the personal cyclone consisted of tests in a sealed glove box for two types of laboratory-generated spores at varying concentrations and for sampling times ranging from 0.08–5.3 hr. The goal of the study was to evaluate the suitability of the cyclone for different analytical techniques (i.e., microscopy, culture, and PCR), using a 15-channel optical particle counter (OPC) and widely used bioaerosol samplers as references. A method to quantify the variability in the between-sampler and between-analysis agreement was used to identify biases, measurements that might be outliers, and relationships that were dependent on the magnitude of the measurements.

MATERIALS AND METHODS

Sample Collection

Fungal Spores

The conidia (asexual spores) of both fungi are globose but differ in diameter with *S. brevicaulis* more than twice the diameter of *A. versicolor* (reported ranges: 5–8 μm and 2–3.5 μm, respectively; aerodynamic diameters: 5.3 and 2.4 μm, respectively) (#66655 and #36009, respectively; American Type Culture Collection, Manassas, Va.).[13–15] The fungi were grown on potato dextrose agar from spore suspensions in sterile distilled water that were spread evenly across the agar surface. Inoculated plates were incubated at room temperature in sunlight until surface growth was dense, spores had matured, and the agar had dried completely (~2 months). Areas of 1–10 cm^2 of dried spores were suctioned through 0.38-mm mesh screens into 37-mm, 2-piece, filter cassettes with cellulose support pads.[16]

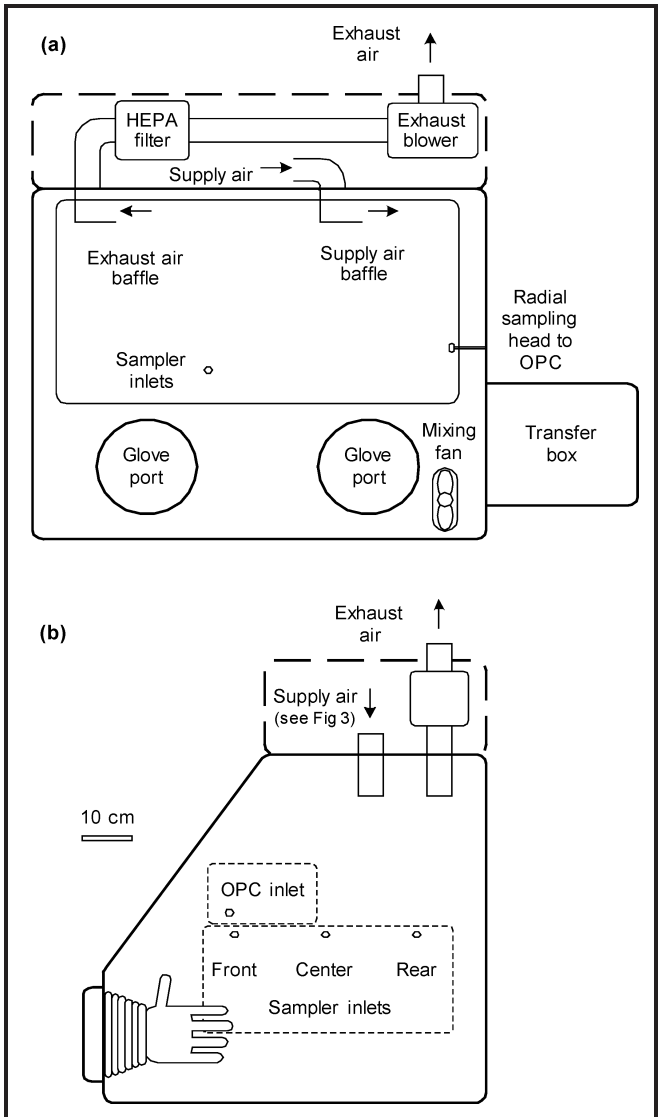

FIGURE 2. Front and side views of glove box showing the positions in which three samplers were placed side by side facing away from the supply air inlet

Aerosol Generation

A 1.3- × 0.8- × 0.9-m fiberglass glove box (model 50350, Labconco Corporation, Kansas City, Mo.) was modified by removal of the high-efficiency particulate air (HEPA) filter at the supply air inlet (Figure 2). Supply air passed a hemispherical perforated metal flow distributor (25 cm^2 with 73 5-mm openings) and charge neutralizer (Nuclear Products Co., El Monte, Calif.) before entering the chamber. Exhaust air passed through a HEPA filter before discharge. The OPC sat on top of the transfer box, outside the chamber, and the radially symmetrical sampling head was inside at the right-hand side of the chamber (Figure 2a). The inlets for the cyclone and two comparison samplers faced away from the supply air baffle. The glove box was enclosed in a walk-in exhaust hood with only the glove ports extending into a room with HEPA-filtered, laminar, ceiling-to-floor airflow.

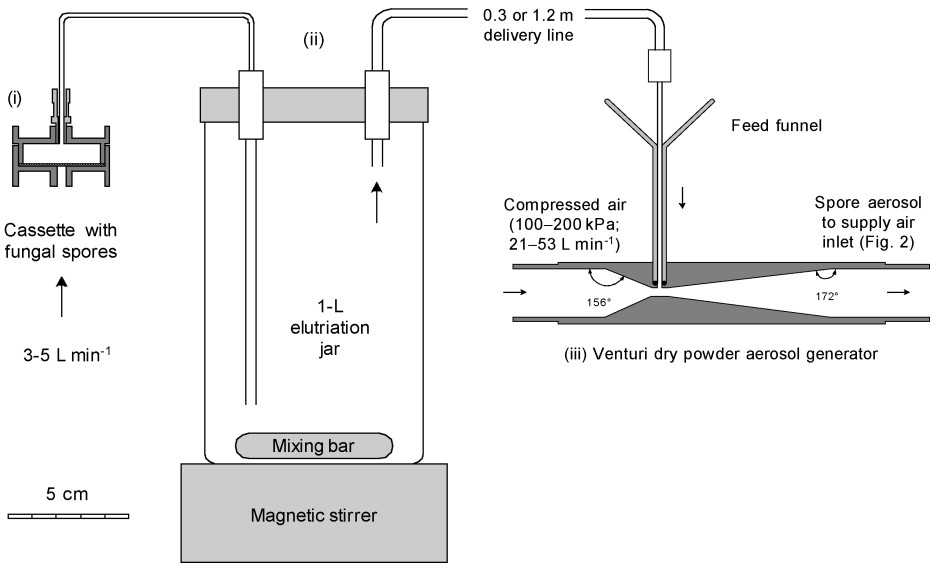

FIGURE 3. Spore delivery system: (i) two-piece cassette with dry fungal spores; (ii) elutriation jar; and (iii) venturi dry powder aerosol generator

A test aerosol was delivered to the glove box by attaching a spore cassette via a stainless steel line (1.6 mm inner diameter) to an elutriation jar and then through the feed funnel of a venturi dry powder disperser (In-Tox Products, Albuquerque, N.M.) (Figure 3).[17] Supply of filtered compressed air to the venturi generator (100–200 kPa) caused an aspiration flow of 2.8–5.1 L min^{-1} through the spore cassette, which suspended spores and delivered them to the test chamber. The aerosol was mixed and large spore clusters were eliminated by passage through 1.0- and 0.5-L glass jars in series for the smaller *A. versicolor* spores; only the 1-L jar and a shorter delivery line were needed for the larger *S. brevicaulis* spores (Figure 3). A small fan mixed the air in the chamber during aerosol delivery (1–2.5 min) and stabilization (1–5 min) but not during sample collection.

Air Samplers

Table I lists the four bioaerosol samplers that were compared, their airflow rates, d_{50} cutpoints, collection media, and assays. All bioaerosol samplers other than the filter collect spores by inertial impaction of particles in an airstream that is directed against a collection surface perpendicular or tangential to the direction of air movement.[18] Vacuum and compressed air were supplied from in-house sources, and airflow was metered with control valves. The cyclone sampler, with a 2-mm wide inlet (Figure 1) (NIOSH, Morgantown, W.Va.), was operated at an airflow rate of 4.0 L min^{-1}, as measured continuously with an in-line mass flow meter (model 4140; TSI Incorporated, Shoreview, Minn.). Particles were collected in conical, screw cap, 1.5-mL MCTs (PGC Scientific, Frederick, Md.) (Figure 1).

TABLE I. Samplers Used in Chamber Comparison Tests

Air Sampler[A]	Total Airflow Rate (L min^{-1})	d_{50}(μm)[B]	Collection Medium	Fungal Measurement
Test bioaerosol sampler				
Cyclone (cyclone) (N = 36)	4.0	1.5	1.5-ml micro-centrifuge tube	Spore, CFU, CE
Reference bioaerosol samplers				
Filter holder (filter) (N = 36)	4.0	—	25-mm, 0.8 μm membrane filter	Spore, CFU, CE
Slit impactor (Air-O-Cell) (N = 18)	15[C]	2.3–2.4	10- × 25-mm glass slide	Spore
Multiple-hole, impactor (N6) (N = 18)	28[D]	0.65	10 ml glycerin gelatin	CFU
Reference real-time particle monitor				
Optical particle counter (OPC) (N = 36)	0.2		15 size channels; >0.30–>20 μm	Particle

[A] Sample size for each of two fungi.
[B] References 10, 19, and 20.
[C] Dilution air was supplied at 11 L min^{-1} so that the sampling rate from the chamber was 4 L min^{-1} (Figure 3).
[D] Dilution air was supplied at 24 L min^{-1} so that the sampling rate from the chamber was 4 L min^{-1} (Figure 4).

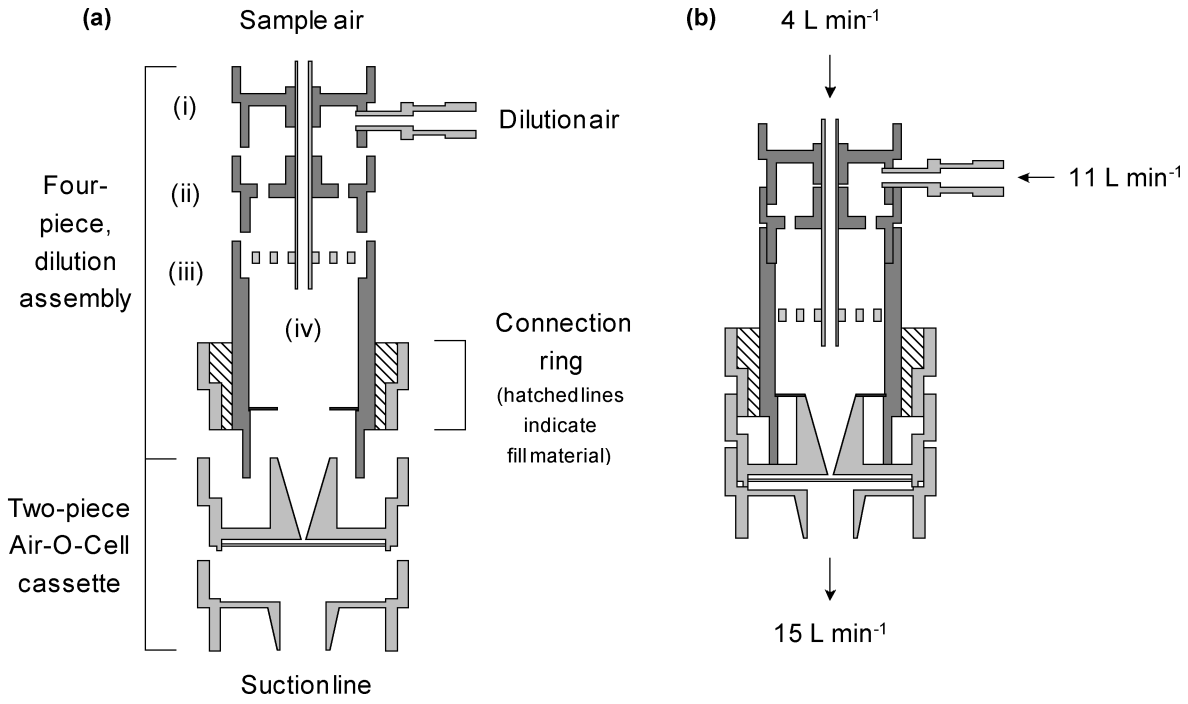

FIGURE 4. 42 mm wide, two-piece, slit impactor sampler with 28.5 mm wide, four-piece, dilution assembly: (a) sampler components expanded: (i) top section of dilution assembly with inlets for sample and dilution air; (ii) middle section (with three, 2-mm holes of which two are shown); (iii) bottom section with face plate and connection ring; and (iv) inlet line with flow distribution screen (with thirty 2-mm holes of which four are shown); and (b) sampler components connected

The filter holder, with an 8-mm wide, 75-mm long, sharp-edged probe (NIOSH, Morgantown, W.Va.); a perforated stainless steel support screen; and a 25-mm, 8.0-μm pore, cellulose ester, support filter (Type SC; Millipore, Billerica, Mass.), also was operated at 4.0 L min^{-1}, measured continuously as above. Samples were collected on polycarbonate, capillary pore, membrane filters (25-mm, 0.8-μm pore, Type ATTP filters; Millipore).

Individual Air-O-Cell slit impactors were operated at 15 L min^{-1} (inlet dimensions: 1.0 $\times$ 14.4 mm) (Zefon International Inc., Ocala, Fla.). Filtered dilution air was delivered to the sampler at a rate of 11 L min^{-1} through three 2-mm holes to match the 4.0 L min^{-1} sampling rates of the cyclone and filter samplers (Figure 4). Dilution and total airflow rates were measured continuously with in-line mass flow meters (models 4140 and 4000, respectively; TSI), and the expected 4 L min^{-1} sampling rate was confirmed with a second model 4140 mass flow meter prior to sample collection. The dilution assembly consisted of 25-mm filter holder parts (Z045BA; Zefon); the top half of an Air-O-Cell cassette from which the center had been removed; and a 25-mm, polypropylene, filter support screen from another filter holder (Swinnex-25; Millipore) as a flow distributor (Figure 4).

The single-stage agar impactor (N6) was operated at 28 L min^{-1} (400 0.25-mm impaction holes) (Thermo Scientific, Waltham, Mass). Filtered dilution air was delivered to the sampler at a rate of 24 L min^{-1} through six 2-mm holes (Figure 5); inlet, dilution, and total airflow rates were measured, as above. Particles were collected on glycerin-gelatin medium (500 mL glycerin and 71 g gelatin per liter distilled water).[21] Glass culture plates (Thermo Scientific) provided the correct jet-to-plate distance when filled with 10 mL of collection medium. All samplers were sanitized by wiping with ethanol after use.

The two optical sensors of the portable aerosol spectrometer provided near real-time measurements of particle number

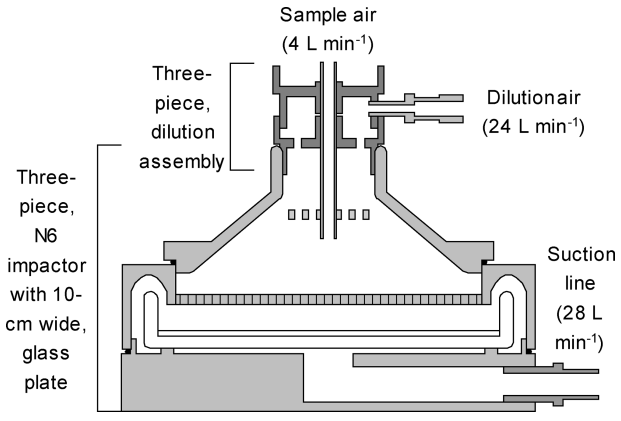

FIGURE 5. Single-stage, 400-hole, agar impactor (sampler components connected) with three-piece dilution assembly: top section; middle section with six, 2-mm holes (of which two are shown); and inlet line with flow distributor as in Figure 4

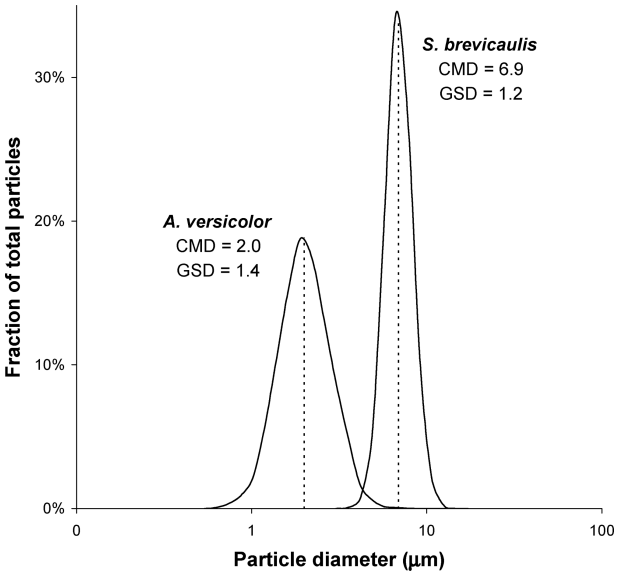

FIGURE 6. Particle size distributions (OPC)

concentration (model 1.108; GRIMM Technologies Inc., Douglasville, Ga.) (channels 1–15: >0.30, >0.40, >0.50, >0.65, >0.80, >1.0, >1.6, >2.0, >3.0, >4.0, >5.0, >7.5, >10.0, >15.0, and >20.0 μm). The OPC was factory calibrated prior to use and performed an automatic system self-test and zero calibration check at each use. Sample collection began when the spore concentration reached a target level, as determined by the OPC, and continued as particle concentration decayed. Target spore concentrations were chosen so that similar numbers of spores would be collected for all sampling times. Particles larger than half the respective count median diameter (CMD; Figure 6) were counted as spores, i.e., $\geq$1.0 μm for *A. versicolor* samples and $\geq$3.0 μm for *S. brevicaulis* (CMD and geometric standard deviations, GSD, calculated with the October 1, 2001, version of the Aerosol Calculator computer program, P.A. Baron, NIOSH, Cincinnati, Ohio). The spore counts for intervals that were approximate multiples of the CMDs were corrected accordingly (e.g., particles 4.0–5.0 and 10–15 μm were multiplied by two for *A. versicolor* and *S. brevicaulis*, respectively); however, these particles accounted for <1% of the total counts.

Six sets of samples were collected for each of six sampling times for each fungus, which allowed positioning of the cyclone, filter, and slit impactor or N6 sampler in all permutations, front-to-rear, relative to each other in the chamber (Figure 2b). The OPC, cyclone, and filter were used in all comparison tests (N = six sampling times × six replicates each = 36 samples for each fungus). The slit impactor was used for the three short-term tests at higher spore concentrations (N = 18) and was replaced with the N6 sampler for the three long-term tests at lower spore concentrations (N = 18). For *A. versicolor*, sampling times were 10, 20, and 40 min and 80, 160, and 320 min (air volume: 0.04–2.5 m^3; mean cyclone concentration: 620–114 000 spore m^{-3}). The 160- and 320-min samples consisted of two and four 80-min runs, respectively. The N6 collection plate was replaced after each 80-min run, but neither the cyclone's MCT nor the filter was changed. Settling velocity for *S. brevicaulis* spores was >10 times that of *A. versicolor*. Therefore, sampling times were shorter due to more rapid deposition of the larger particles: 5, 10, and 15 min and 20, 25, and 30 min (air volume: 0.02–0.12 m^3; mean cyclone concentration: 4470–176 000 spore m^{-3}, respectively).

Sample Analysis

All analyses, other than N6 cultures, were done by a laboratory accredited by AIHA in environmental microbiology and licensed by the U.S. Environmental Protection Agency for their PCR procedure (www.epa.gov/nerlcwww/moldtech.htm). MCTs, filters, and Air-O-Cell cassettes were held at room temperature until delivered overnight to the laboratory within 30 days of collection.

For the Air-O-Cell samples, spores were counted with a light microscope at 600× magnification (Olympus model CX41, Melville, N.Y.). Spore analysis was based on examination of 25% of the total sample. Concentration was based on counting 3600–4300 *A. versicolor* and 1600–4200 *S. brevicaulis* spores.

Culture of the N6 samples was done immediately following sample collection. The glycerin-gelatin medium was warmed to 40°C and diluted with sterile distilled water before inoculation of MEA in triplicate and incubation at room temperature for 7–10 days. The average count for the dilution series that yielded the highest countable number of CFUs was recorded (CFU analysis: *A. versicolor*, 0.5%–1.5% of total sample, 20–40 CFU counted; *S. brevicaulis*, 1.0%–15% of total sample, 80–110 CFU counted).

Spores were washed from the cyclone MCTs using 1 mL of ultrapure water with 0.05% polyoxyethylene sorbitan monooleate 80 (Tween 80) and vortexed for 15–30 sec. Spores were washed from membrane filters using 5 mL of wash solution that was centrifuged and resuspended to 1 mL. Recovery efficiencies were determined for 10 μL of a known spore suspension pipetted in triplicate into MCTs or onto filters (average efficiency = 88% and 90%, respectively). Dilutions of cyclone and filter suspensions were made as needed to obtain countable numbers of spores, CFUs, and CEs.

Spores were counted, as above, for 50 μL of the cyclone and filter washes, which were spread on glass slides with imprinted counting grids and allowed to dry on a slide warmer for 5 min (spore analysis: *A. versicolor*, 2.5%–5.0% of total sample, 40–390 spores counted; *S. brevicaulis*, 5%–10% of total sample, 30–500 spores counted). Culturable spores were analyzed, as above, for 100 μL of the cyclone and filter washes (CFU analysis: *A. versicolor*, 0.1%–1% of sample, 12–140 CFU counted; *S. brevicaulis*, 1%–10% of sample, 40–85 CFU counted).

Species-specific CE were determined using quantitative real-time PCR (qPCR)[22,23] for 500 μL of the cyclone and filter samples (CE analysis: *A. versicolor* and *S. brevicaulis*, 50% of total sample, 2100–60 000 and 170–6000 CE detected,

respectively). Cell suspensions were extracted by a rapid bead-milling method.[24] CE were determined from standard calibration curves based on counting *A. versicolor* and *S. brevicaulis* spores in suspension. An internal reference was introduced prior to DNA extraction to account for extraction efficiency and PCR inhibition.[22,23] All primer and probe sequences used in the assays are available at www.epa.gov/nerlcwww/moldtech.htm.

Previously determined coefficients of variation (CVs) for spore count, culture, and PCR analyses were 3%, 16%, and 25%, respectively. Detection limits were one spore, CFU, or CE (Air-O-Cell: 2–200 spore m^{-3}; N6: 27–5000 CFU m^{-3}; cyclone and filter: 8–1000 spore m^{-3}, 40–25 000 CFU m^{-3}, and 1–50 CE m^{-3}). Quantitation limits were 10 times the detection limits.

DATA ANALYSIS

The natural logarithms (ln) of the concentration measurements were evaluated because they were distributed more normally. Bioaerosol sampler concentrations were normalized to their respective OPC measurements, and analysis of variance was used to evaluate the effect of sampler placement in the front, center, or rear of the chamber. Regression was used to adjust for position effect. Agreement between two sets of measurements often is presented as a scatter plot in combination with regression and correlation. Correlation was used to measure the strength of the relationships between samplers and analyses, but this means of summarizing data depends on the range and distribution of paired measurements and ignores any systematic bias that may exist. Bias was identified as regression slopes (β) different from one, and the nature of the bias was examined using a procedure to identify and quantify between-method disagreement.

Bland-Altman plots have become popular in clinical chemistry and have been used to evaluate instruments, such as spirometers.[25–28] The approach assumes that method-comparison studies aim to quantify disagreements between individual measurement methods, to identify biases and outliers, and to evaluate relationships that may depend on the magnitudes of the measurements.

Several metrics were considered, e.g., the difference between paired measurements, the difference as a percentage of the average (A) of paired measurements, and the ratio (R) of paired measurements. All metrics agreed, and R was chosen as the most readily understood for all comparisons. Values of R were plotted against A, the line of best agreement was determined by regression of R on A ($\hat{R} = \alpha + \beta A$), bias was estimated as the mean ratio ($\overline{R}$), and variation around $\overline{R}$ was estimated by the standard deviation (SD) of R. Upper and lower limits of agreement (LA) identified the intervals containing 95% of R. Rs and SDs could vary with concentration. Therefore, bounds were calculated not as parallel lines (i.e., $\overline{R} \pm$ 2 SD) or as 95% confidence intervals (CI) wider at both extremes than in the center.

Instead, the LA were determined by modeling the variability in the SDs directly as a function of the measurement level (estimated by A) using a method based on absolute residuals ($|r|$) from a fitted regression line (where $r = R - \overline{R}$). Predicted residuals ($\hat{r}$) were determined by regressing r on A, and the LA were $\hat{R} \pm t_{n(2.5\%)}\sqrt{\pi / 2\hat{r}}$, where $t_{n(2.5\%)}$ was the critical value from the Student's t-distribution for 18 or 36 samples.[26] CIs on the LA identified measurements that might be considered outliers.[27]

This comparison highlighted features such as whether the cyclone (or one analysis) over- or underestimated another (i.e., $R > 1$ or < 1, respectively); whether R varied with sampling time and spore concentration (i.e., $\beta \neq 0$ for R on A plots); and whether variation around $\overline{R}$ was the same across all spore concentrations or greater at one end of the distribution (i.e., upper and lower LA parallel or not, respectively). Two methods could be considered interchangeable if R clustered tightly around one with β close to zero, and, if desired, one measurement could be converted to the other if any observed deviations from this pattern were consistent.

RESULTS

Particle Size Distributions

The majority of *A. versicolor* spores were observed in the 1.0–1.6, 1.6–2.0, and 2.0–3.0 μm ranges of the OPC (median diameters: 1.3, 1.8, and 2.5 μm, respectively) and *S. brevicaulis* spores in the 4.0–5.0 and 5.0–7.5 μm ranges (median diameters: 4.5 and 6.3 μm, respectively). Respective CMDs of 2.0 and 6.9 μm agreed with previous observations of spore dimensions; and respective GSDs of 1.4 and 1.2 indicated that the aerosols were not far from what has been considered monodisperse (i.e., GSD < 1.2)[29] (Figure 6). Figure 7 also illustrates that both aerosols consisted predominantly of single spores (>70%) whether examined on a direct impaction surface (Air-O-Cell slides) or after suspension in liquid (cyclone and filter washes). One and two *A. versicolor* conidiophores (structures on which conidia are formed) were reported for two Air-O-Cell samples but not for cyclone or filter samples.

Comparability of Air Samplers

Sampler performance was comparable for all locations in the chamber, with the exception of *A. versicolor* CFU concentrations, which were higher when the N6 sampler was in the rear relative to the center of the chamber ($p = 0.03$) and when the cyclone was in the rear relative to the front ($p = 0.04$). The latter finding also was significant with *S. brevicaulis* CFU ($p = 0.01$).

Sampler measurements were significantly positively correlated, but there was bias between the cyclone and the OPC as well as the bioaerosol samplers (i.e., $\beta \neq 1$) (Table II and Figure 8). Plots of R vs. A revealed that cyclone:filter agreement was fairly uniform across spore concentration (Figure 9). That is, the upper and lower LA were approximately parallel for all cyclone:filter comparisons although the LA tended to be somewhat wider for longer samples at lower

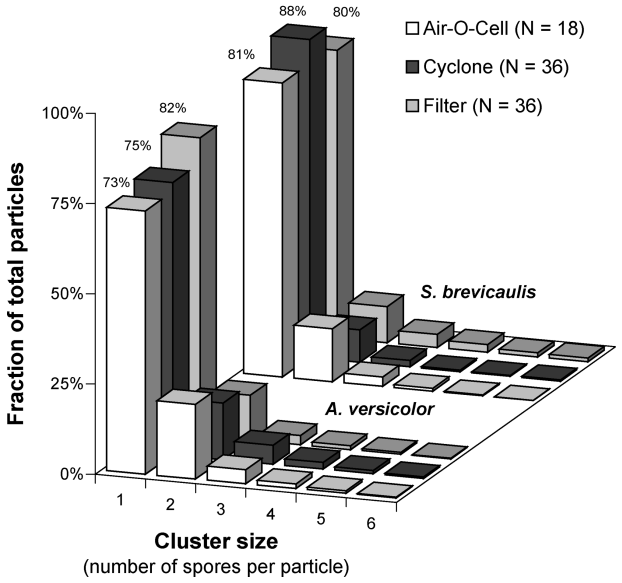

FIGURE 7. Average spore-cluster distributions (bioaerosol samplers)

A. versicolor concentrations when multiple 80-min runs were combined (Figure 9a).

Similar comparisons for cyclone:Air-O-Cell spore, cyclone:N6 CFU, and cyclone:filter CE measurements also showed good agreement, i.e., no observations exceeded the 95% CI on the LA (data not shown). Agreement was better at higher concentrations (R closer to one), but on average, the cyclone underestimated the comparison sampler (i.e., $\overline{R} < 1$) except for *A. versicolor* cyclone: N6 CFU ($\overline{R} = 1.04$) (Table II). The slope for the latter comparison was the only one that did not differ significantly from zero; otherwise, R varied with fungal concentration ($\beta \neq 0$) (Figure 9).

R and A also were calculated for paired spore, CFU, and CE counts rather than concentrations for the cyclone and filter samplers to remove the effect of sampling time (data not shown). Calculation of cyclone:filter count R reduced the horizontal spread of the data (i.e., the points overlapped more because similar numbers of spores were collected for all sampling times), and the LA were more parallel. Therefore, differences in the performance of the cyclone relative to the filter depended on the number of spores that were collected, and disagreement was greater for lower spore numbers regardless of the time period over which they had been collected.

Comparability of Spore Count, Culture, and PCR Analyses

Correlations, R, and A for paired CFU:spore, CFU:CE, and CE:spore measurements for the cyclone and filter samplers were calculated (Table III) and plotted, as above. Agreement between the assay methods was good, i.e., no observations exceeded the 95% CIs on the LA (data not shown). However,

TABLE II. Correlation Coefficients and Mean Ratios ($\overline{R}$) of Paired Cyclone and Comparison Sampler Measurements (with 95% CIs)

	A. versicolor		*S. brevicaulis*	
Comparison–Samplers	Correlation	$\bar{R}$	Correlation	$\bar{R}$
		Spores		
Cyclone:OPC (N = 36)	**1.00** (0.99–1.00)	0.75 (0.53–0.96)	**0.89** (0.80–0.95)	0.87 (0.72–1.02)
Cyclone:Filter (N = 36)	**0.99** (0.98–1.00)	0.86 (0.67–1.04)	**0.99** (0.99–1.00)	0.89 (0.78–0.99)
Cyclone:Air-O-Cell (N = 18)	**0.92** (0.84–0.96)	0.89 (0.84–0.95)	**0.88** (0.78–0.94)	0.91 (0.83–1.00)
		Colony-Forming Units (CFU)		
Cyclone:OPC (N = 36)	**0.99** (0.98–0.99)	0.89 (0.78–0.99)	**0.92** (0.85–0.96)	0.87 (0.76–0.99)
Cyclone:Filter (N = 36)	**0.99** (0.99–1.00)	0.96 (0.88–1.04)	**1.00** (0.99–1.00)	0.97 (0.93–1.00)
Cyclone:N6 (N = 18)	**0.86** (0.73–0.92)	1.04 (0.95–1.13)	**0.79** (0.62–0.89)	0.97 (0.89–1.04)
		Cell Equivalents (CE)		
Cyclone:OPC (N = 36)	**0.99** (0.98–1.00)	0.84 (0.74–0.93)	**0.82** (0.67–0.91)	0.85 (0.63–1.07)
Cyclone:Filter (N = 36)	**0.98** (0.96–0.99)	0.87 (0.77–0.97)	**1.00** (1.00–1.00)	0.88 (0.78–0.99)

Note: Statistically significant correlations in **bold**.

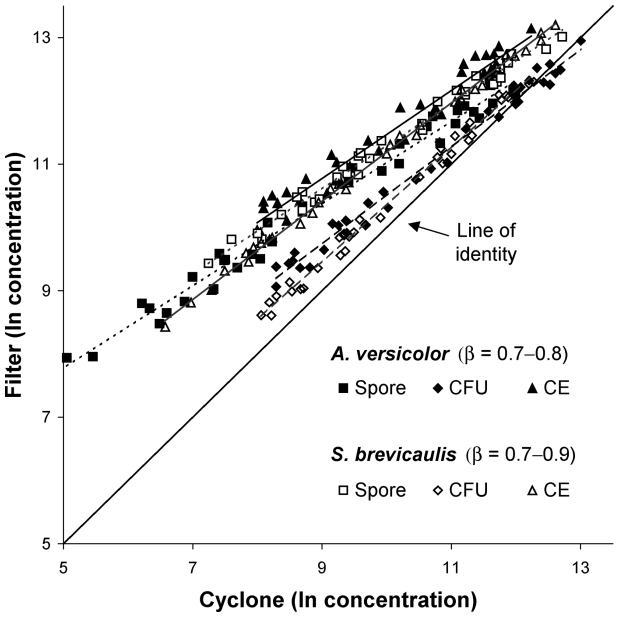

FIGURE 8. Examples of the comparability of paired cyclone and filter samples (all slopes, β, significantly below one; correlations in Table II) (N = 36 for each comparison)

bias was identified, i.e., $\overline{R} \neq 1$, with the exception of the *S. brevicaulis* CFU:spore comparison for the cyclone sampler. *R* varied with fungal concentration, i.e., $\beta \neq 0$, with the exception of the *A. versicolor* CFU:CE comparison for the cyclone and the *S. brevicaulis* CFU:CE comparison for the filter.

R and *A* again were calculated for count data to remove the effect of sampling time (data not shown). As for the cyclone:filter comparison, the magnitude of the differences between the analytical methods also depended on the number of spores, CFUs, or CEs that were detected, and disagreement between the analyses was greater for lower numbers for both the cyclone and filter samplers.

DISCUSSION AND CONCLUSIONS

Some lack of agreement between measurement methods is inevitable. Correlation was used to measure the strength of relationships, and a graphical procedure that is simple to perform and interpret was chosen to quantify disagreements that were observed between the cyclone and three standard bioaerosol samplers and among three assay methods.

Agreement between the cyclone and comparison samplers was somewhat better for CFU than for spore or CE assays for both *A. versicolor* and *S. brevicaulis*, i.e., $\overline{R} = 0.89$–1.04 vs. 0.75–0.91 and CIs on $\overline{R}$ were narrower (Table II). Agreement among the three analytical methods tended to be better for *S. brevicaulis* than for *A. versicolor* and better for filter than cyclone samples for *A. versicolor* (Table III). Several factors may have contributed to the differences that were observed. *A. versicolor* would have been collected less efficiently than *S. brevicaulis* because spore diameter for the former was nearer the cyclone's d_{50} (Table I). The smaller spores also may have been more difficult to count accurately by microscope explaining the higher CFU:spore and CE:spore concentrations for this species (Table III).

PCR can measure DNA in spores, spore fragments, and hyphal fragments such as conidiophores.[30] Therefore, CE measurements would exceed spore concentrations if the PCR analysis detected DNA in cell fragments as well as intact spores. However, harvesting spores through a filter appears to have reduced the amount of mycelial debris in the dried spore preparations (few conidiophores were observed), but

TABLE III. Correlation Coefficients and Mean Ratios ($\overline{R}$) of Paired Analyses (with 95% CIs; N = 36)

	Cyclone		Filter	
Comparison–Analyses	Correlation	$\overline{R}$	Correlation	$\overline{R}$
		A. versicolor		
CFU:spore	**0.98**	1.21	**0.98**	1.06
	(0.95–0.99)	(0.94–1.48)	(0.96–0.99)	(0.99–1.14)
CFU:CE	**0.90**	1.06	**0.89**	0.96
	(0.81–0.95)	(0.92–1.21)	(0.80–0.95)	(0.86–1.06)
CE:spore	**0.89**	1.15	**0.87**	1.11
	(0.79–0.94)	(0.78–1.51)	(0.76–0.93)	(0.94–1.29)
		S. brevicaulis		
CFU:spore	**0.94**	**1.01**	**0.94**	0.92
	(0.88–0.97)	(0.89–1.12)	(0.88–0.97)	(0.84–0.99)
CFU:CE	**0.92**	1.04	**0.93**	0.95
	(0.84–0.96)	(0.87–1.21)	(0.86–0.96)	(0.86–1.04)
CE:spore	**0.92**	0.97	**0.92**	0.97
	(0.84–0.96)	(0.82–1.13)	(0.85–0.96)	(0.87–1.13)

Note: Statistically significant correlations in **bold**; $\overline{R}$ in bold not significantly different from one.

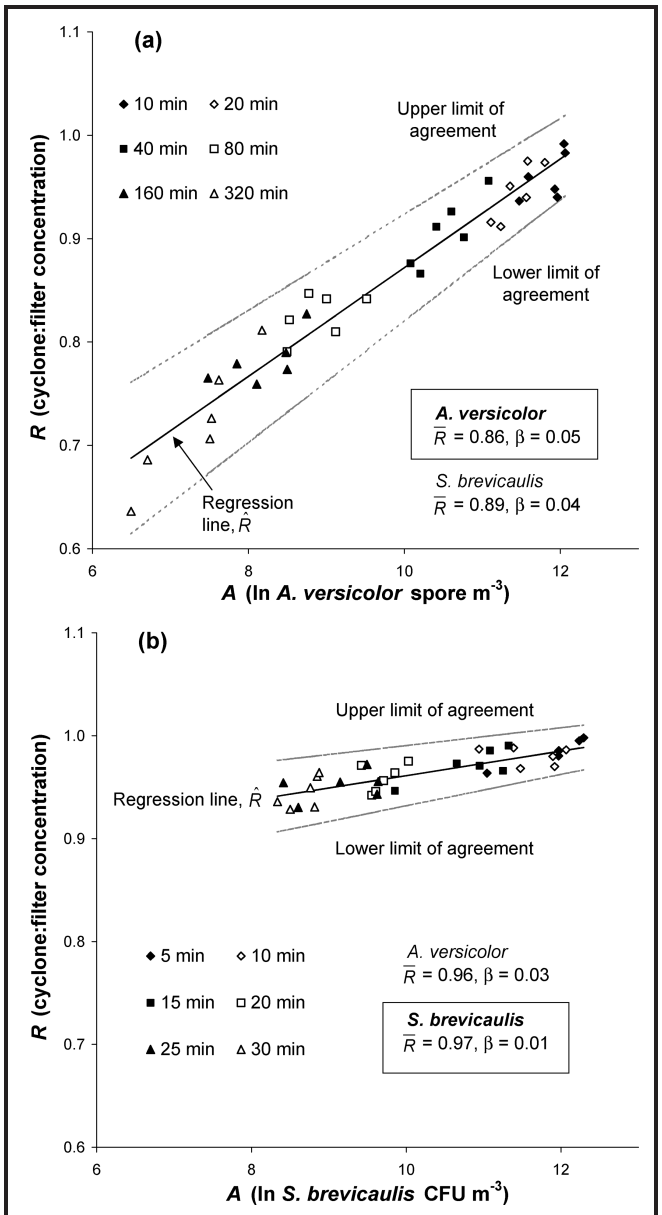

FIGURE 9. Examples of ratio (R) vs. average (A) plots illustrating the comparability of paired cyclone and filter samples (Table II) (N = 6 for each sampling time): (a) *A. versicolor* spore concentration (average ratio, $\overline{R}$, and slope, β, for *S. brevicaulis* spores included for comparison); and (b) *S. brevicaulis* CFU concentration ($\overline{R}$ and β for *A. versicolor* CFUs included for comparison)

fragments smaller than spores may have passed through the elutriation chambers.

The cyclone was operated without a backup filter because this would have required the use of a more powerful air mover and would have doubled the analytical costs. Chen et al.[10] found that 65–78% of 2-μm particles were collected in the MCT, with 11–16% in the attachment and 10–18% on a backup filter, which may explain why cyclone measurements generally were lower than the other samplers (Table II). Therefore, washing the cyclone inlet and outlet and using a backup filter to collect particles otherwise lost to bounce should be considered. In addition, coating the MCT surface may have improved particle retention,[9] although Chen et al.[10] found no difference in cyclone performance for various MCTs with or without a polyethylene glycol coating.

The primary use of the OPC was as a real-time indicator of particle concentration and approximate size distribution. To minimize the distance particles traveled to the detector, the OPC inlet was close to a side wall and distant from the bioaerosol samplers, which may in part explain the poorer agreement between the cyclone and OPC relative to agreement with the more centrally placed bioaerosol samplers ($\overline{R}$ = 0.75–0.89 vs. 0.86–1.04, Table II).

The bioaerosol samplers were aligned in two dimensions, but the front-to-rear placement in the chamber introduced a potential source of error (Figure 2b). Positioning samplers in all configurations relative to each other helped to balance potential bias due to placement, and regression was used to adjust for position effect. The need to reduce the airflow rate of the N6 sampler involved modifying its inlet (Figure 5). Chamber height was greatest in the rear of the glove box (Figure 2b), and the upward-facing inlet of the dilution assembly may have allowed over-collection of spores not in the original still air volume contributing to the significantly higher CFU concentrations in this position for *A. versicolor*.[31] This effect was not observed for the shorter duration tests, i.e., the Air-O-Cell sampler with the same diluter inlet and tests with the *S. brevicaulis* spores. Immediate culture of the N6 samples would not explain a difference due to N6 position but may have contributed to disagreement with the cyclone sampler in the comparisons based on CFU concentration (Table II). An explanation for measurement of higher CFU concentrations in the rear of the chamber for the cyclone sampler is not obvious because no significant position differences were observed for spore or CE measurements.

The magnitude and variability of the observed disagreements could be related to sample size and concentration range, limiting the ability of comparisons with the Air-O-Cell and N6 samplers to detect differences relative to the filter sampler (N = 18, three concentrations vs. N = 36, six concentrations). The wider concentration range for *A. versicolor* may have contributed to the generally higher correlations relative to *S. brevicaulis* (0.92–1.00 vs. 0.79–1.00, Table II).

An advantage of the cyclone and filter samplers is that all three analyses were performed on the same spore suspensions, which could be diluted as needed to obtain appropriate numbers of spores, CFUs, and CEs. That the spore suspensions for these samplers were analyzed identically may have contributed to the good agreement between the cyclone and filter.

The graphical comparisons revealed that differences between samplers and analyses depended on the number of spores that were collected and that disagreement was greater and more variable for lower spore numbers regardless of the time period over which they were collected. Assuming that the reliability of a measurement depended on the amount of analyte detected, PCR would be expected to give the best estimate (based on analysis of half the filter and cyclone

samples; 170–60 000 CE), followed by spore counting (2.5–25%; 30–4300 spores) and culture (0.5–15%; 20–110 CFUs). However, the repeatability of PCR measurements was poorer than the other assays (CV = 25% vs. 3% and 16% for PCR, spore counting, and culture, respectively). Therefore, the spore count data may have been the most reliable because least variable, based on analysis of not less than 2.5% of the sample and counting of not fewer than 30 spores, and not dependent on cell viability (although possibly affected by spore size). Godish and Godish[32] also observed greater variability at lower spore densities and recommended that variability could be reduced by examination of a larger fraction of low-density samples.

The cyclone proved to be a convenient device that performed well in these chamber tests with two, approximately spherical fungal spores of different sizes. The cyclone that previously was evaluated for collection of nonbiological particles has been shown capable of accurately collecting laboratory-generated fungal spores for analysis by microscopy, culture, and PCR. The next series of evaluations will involve collection of ambient airborne spores (some of which occur at much lower air concentrations than in these chamber tests), longer sampling times (7–19 hr), and comparison to two other bioaerosol samplers that were too large to evaluate in the chamber (i.e., a different slit impactor and another cyclone sampler).[11]

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