

## **THE BI-AIR FILTER CASSETTE: SAMPLING AIRBORNE FUNGAL SPORES IN A SETTLING CHAMBER**

Work performed by:

Joe Spurgeon, Ph.D., CIH    Indoor Health Associates    [cih@bi-air.com](mailto:cih@bi-air.com)

### **ABSTRACT**

The Bi-Air (BA) dual-trace 25 mm filter cassette was described, and its performance for the collection of *Aspergillus/Penicillium* (*Asp/Pen*) and *Stachybotrys* type spores in a clam-air settling chamber was compared to that of the Air-O-Cell (AOC) cassette.

The BA produced duplicate sample traces, the sample traces were confined to two small, well-defined areas on the filter, and the small size of the sample traces made direct microscopic examination of the samples possible without requiring a sample transfer step prior to analysis. The BA was similar in size to the AOC, and had the same ease of use and analysis. However, the BA did not have the limitations of a slit impaction sampler for the collection of fungal spores.

The average ratio of BA to AOC concentrations for *Asp/Pen* spores were 2.0 and 1.8 at airflow rates of 1 lpm and 2 lpm, respectively. The ratio decreased to 1.5 at an airflow rate of 3 lpm. The average ratios for *Stachybotrys* varied between 1.0 and 1.5 for the three airflow rates, with an average of 1.1.

Practical implications.

The BA, a 25 mm filter cassette, can be used for the routine collection and analysis of airborne fungal spores. It produced duplicate sample traces, the sample traces were confined to two small, well-defined areas on the filter, and the small size of the sample traces made direct microscopic examination of the samples possible without requiring a sample transfer step prior to analysis. The BA was more efficient at retaining small *Asp/Pen* type spores compared to the AOC, and had the same ease of use. However, the BA did not have the limitations of a slit impaction sampler for the collection of fungal spores.

## INTRODUCTION

### Background

The desirable characteristics of a bioaerosol sampler have been described by several authors. Macher included low airflow rate, low transmission losses, size-selective sampling efficiency, and ease of handling as desirable characteristics.<sup>(1)</sup>

Aizenberg et al mentioned low induced turbulence at the inlet, the use of conductive materials to minimize electrical effects, and uniform particle deposition on the collection medium as desirable characteristics.<sup>(2)</sup>

Chen et al listed a number of other characteristics that were desirable in a bioaerosol sampler.<sup>(3)</sup> These included small size; disposable collection medium; ability to collect area and personal samples; ability to collect both short-term (“grab”) and long-term(TWA) samples; applicable to a wide range of concentrations; high aspiration efficiency; high collection efficiency; simple analysis without sample transfer; and the ability to support a variety of analytical methods.

Although not specifically mentioned by these authors, the following characteristics may also be desirable in a sampler designed to collect airborne fungal spores:

- a small, bounded sample trace with well-defined boundaries;
- a sampler design that produces duplicate sample traces; and
- the ability to vary inlet size with airflow rate.

Slit impaction samplers are one of the most commonly used methods for collecting airborne fungal spores.<sup>(3, 4)</sup> They are simple to use, easy to analyze, and cost effective. However, slit impaction samplers have several important limitations with reference to the above lists of desirable characteristics, including:<sup>(3, 5)</sup>

- relatively high airflow rates that are generally maintained in a narrow range, limiting their adaptability to varying conditions and spore concentrations;
- generally limited to the collection of short-term “grab” samples of 15 minutes or less, limiting their utility for collecting personal samples and extended-period samples; and
- spore retention is affected by particle cutoff size and spore morphology, resulting in variability between the retention of spore types.

In addition, the gap between the sampler inlet and the impaction surface in slit impaction samplers results in a diffuse sample trace. The gap allows the spores to become spread over an unbounded area that can be two to three times the width of the inlet.<sup>(2)</sup> This characteristic often makes it more difficult for the analyst to detect all the deposited spores.

## Bi-Air Chamber Tests

Filter samplers are not subject to these limitations, and therefore offer the potential for improved performance for the collection of airborne fungal spores. In addition, they possess a number of the characteristics desirable in a bioaerosol sampler, including the ability to collect area and personal samples; high collection efficiency; the ability to collect both short-term (“grab”) and long-term samples; applicable to a wide range of concentrations; and the ability to support a variety of analytical methods.

Filter cassettes have been used for the collection of airborne fungal spores for many years.<sup>(6-10)</sup> However, the large surface area of filters has limited their utility for the quantitative analysis of spores by standard bright field microscopy.<sup>(11)</sup> Studies that have evaluated filters as the collection medium for airborne spores either estimated the spore concentration using a small area of the filter, or the analysis required an intermediate sample transfer step such as washing the filter or staining prior to epifluorescence microscopy.<sup>(2, 6-8)</sup>

This factor has resulted in the limited use of filter samplers by indoor air quality professionals. Therefore, a filter sampler that produced a bounded, well-defined sample trace which was sufficiently small to analyze by direct microscopic examination, without requiring a sample transfer step, would be desirable.

### **Purpose**

The first objective of the study was to describe the physical characteristics of the Bi-Air (BA) filter cassette.<sup>(12)</sup> The BA produces duplicate sample traces, the sample is confined to a small, well-defined area on the filter, and the size of the inlet may be varied to maintain a constant inlet velocity with variable airflow rates. The BA was described with respect to the characteristics that have been identified as being desirable in a bioaerosol sampler.

The second objective was to demonstrate that filter samples collected with the BA could be analyzed quantitatively, using standard bright field microscopy, without requiring an intermediate sample transfer step. The hypothesis was that the small, sharply defined sample trace produced by the BA would allow filtration to be used for the direct quantitative analysis of airborne fungal spores.

The third objective was to compare the performance of the BA for the collection of *Aspergillus/Penicillium* (*Asp/Pen*) and *Stachybotrys* type spores to that of the Air-O-Cell (AOC) slit impaction cassette. Comparisons were made by collecting side-by-side samples in a clam-air settling chamber under conditions typical of indoor environments. The hypothesis was that the BA filter cassette would provide improved performance relative to a typical slit impaction sampler for the retention of small fungal spores.

## METHODS

### Bi-Air Cassette

The BA cassette was a 25 mm filter cassette with an overall length of 4.1 cm and a diameter of 3.0 cm. It was constructed of electrically dissipative polystyrene containing 4 % carbon.

Figure 1 is a side view of the BA, showing one of the four sample inlets placed at 90° around the inlet cowl. The BA was prepared for sampling by turning the cap to align the slots in the side of the cap to those in the side of the cowl. The inlet cowl had a diameter of 15.9 mm and a length of 12.7 mm. The slotted cowl and cap formed an enclosed chamber.



Figure 1. Bi-Air Cassette: Side view of the BA showing one of the four variable-width 3 mm x 3 mm sample inlets located in the side of the cowl.

When fully open, each inlet was a 3-mm square with an individual area of 9 mm<sup>2</sup> and a maximum total area for the four inlets of 0.36 mm<sup>2</sup>. Samples were collected by positioning the cassette in a vertical orientation and leaving the inlets fully open.

### Sample Traces

Figure 2 is a photo of the interior of the cassette cowl, showing the position of the duplicate sample traces.



Figure 2. Bi-Air Cassette: Interior of the cowl showing the 1.7 mm x 5.5 mm dual sample traces with a total exposed filter area of 18.7 mm<sup>2</sup>.

## Bi-Air Chamber Tests

The 25 mm filter was covered by a plastic disc containing two rectangular slits, each having dimensions of 1.7 mm x 5.5 mm. Each of the two sample traces were separated by 4 mm and had an area of 9.35 mm<sup>2</sup>, with a total exposed filter area of 18.7 mm<sup>2</sup>. The area of the sample trace was restricted to the exposed area of the filter by compression ridges on the base of each sample slit. The ridges compressed the filter, (1) confining the spores within the sample trace, and (2) allowing the outline of the sample traces to be seen when the filter was removed.

### Filter Pore Size

Six BA cassettes containing MCE filters were attached to the sampling ports in the settling chamber. The cassettes were arranged in the chamber in an alternating pattern, with two cassettes each containing filters with pore sizes of 1.2 µm, 3 µm and 5 µm. The airflow rate for each BA was adjusted to 2 lpm, and 30-minute samples were collected. The relative concentrations of *Asp/Pen* (about 10,000 spores/m<sup>3</sup>) and *Stachybotrys* (about 600 spores/m<sup>3</sup>) spores detected using the three pore sizes were used to evaluate the effect of filter pore size on spore retention.

### Airflow Rate

A BA was attached to sampling ports 1, 3 and 5 in the settling chamber. The airflow rates of the three BA were adjusted to 1, 2 and 3 lpm, respectively. Three AOC were attached to sampling ports 2, 4 and 6, and the airflow rate adjusted to 15 lpm. The three BA samples were collected for 30 minutes. The three AOC samples were collected in series, with each AOC sampling for 10-minutes. The concentrations detected with the BA were compared to the average AOC concentration for the 30-minute period. The concentration of *Asp/Pen* type spores in the chamber ranged between 3,000 and 30,000 spores/m<sup>3</sup>, and *Stachybotrys* concentrations ranged between 100 and 1,200 spores/m<sup>3</sup> during the series of six tests.

### Settling Chamber

The settling chamber illustrated in Figure 3, and the details of the test protocol used to compare the two samplers, have been described.<sup>(13)</sup> The chamber was a hexagonal plywood box with an overall height of 122 cm and an interior volume of approximately 2,300 liters. Six sampling ports were located in the bottom of the chamber in a circular pattern. The sampling ports allowed six side-by-side samples to be collected during each test. The CV for concentrations measured at the six sampling ports were 9 % for an *Asp/Pen* type spore concentration of about 10,000 spores/m<sup>3</sup>. In addition, the test section of the chamber was under clam-air conditions during the comparison tests, with air velocities less than 30.5 cm/sec.<sup>(13)</sup>

## Bi-Air Chamber Tests

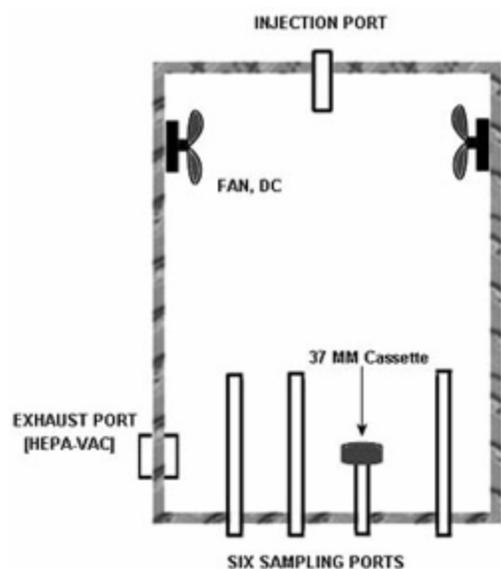


Figure 3. Schematic diagram of the hexagonal calm-air settling chamber.

### Sample Analysis

Following sample collection, the AOC cassette was opened and the adhesive strip was removed. A drop of liquid adhesive was placed on a glass slide, and the bottom of the sample slide was placed on the liquid adhesive. The sample was stained with lactophenol cotton blue and covered with a cover slip. A total of 11 traverses were analyzed, or about 22 % of the sample trace. This was equivalent to an analyzed sample volume of 33 liters for a 10-minute sample. The AOC samples were analyzed by beginning the analysis near one end of the sample trace, but within the sample area. Every other traverse was analyzed until a maximum of eleven traverses had been analyzed.

A BA cassette containing a mixed cellulose ester (MCE) filter (Millipore, Bedford, MA) was attached to the sample port in a vertical position using a short length of PVC tubing. The airflow rate of the pump was then adjusted to 1, 2 or 3 lpm using a 60 mm rotameter with a range of 0-5 lpm. A maximum sample collection time of 30 minutes was used. Following sample collection, the cassette was opened and the filter and pad were placed on a clean surface. The two sample traces were separated by cutting the filter in half using an Xacto Knife with a ½ inch blade. One sample trace was placed on a glass slide, and the slide inserted into a Quick Fix® acetone vaporizer (EMS, Charleston, SC) to collapse and clear the MCE filter. The slide was removed, and the sample was stained with lactophenol cotton blue and covered with a cover slip. A total of 10 traverses were analyzed, or about 52 % of the sample trace. This was equivalent to an analyzed sample volume (per sample trace) of 7.7 liters at 1 lpm, 15.5 liters at 2 lpm, or 23 liters at 3 lpm. The BA samples were analyzed by starting at one edge of the sample trace and counting every traverse until a maximum of ten traverses had been analyzed. The first traverse, adjacent to the edge of the trace, was not analyzed.

## Fungal Spores

Fungi collected and identified during field investigations were cultured on malt extract agar (MEA). The spores from the culture plates were collected with sterile swabs and transferred into 30 ml plastic bottles containing 2 ml of 70 % isopropanol (USP). The test solution was prepared by transferring a 1.0 ml aliquot from each of the *Aspergillus/Penicillium* (*Asp/Pen*) and *Stachybotrys* stock solutions into 8.0 ml of alcohol in another 30 ml plastic bottle. A number of 4 mm diameter glass beads were added to the test solution in order to mix settled spores. The stock solution was stored at room temperature and used over a period of about four weeks.

Fungal spores were identified and counted by bright field microscopy at 600X magnification. Analyses were performed using a Nikon Alphaphot microscope (Nikon America, NY, NY) equipped with a 10x eyepiece and a planachromat 60x objective. The spherical to ovoid *Asp/Pen* type spores were smooth and had a diameter of about 3  $\mu\text{m}$  as measured by a Walton Beckett graticule. They were similar in appearance to *P. Chrysogenum*. Many of the *Stachybotrys* spores, 4-6  $\mu\text{m}$  x 7-12  $\mu\text{m}$ , were translucent and had a smooth surface typical of young spores.

## RESULTS

### Sample Trace

The relative appearances of the sample traces produced by the AOC and the BA were compared at 100X magnification. The corner of a typical AOC sample trace at 100X magnification is illustrated in Figure 4. The edges of the sample trace were diffuse and its boundaries were not sharply defined. The area in which *Asp/Pen* type spores were detected covered 30-40  $\text{mm}^2$ , or approximately three times the width of the inlet slit.

## Bi-Air Chamber Tests

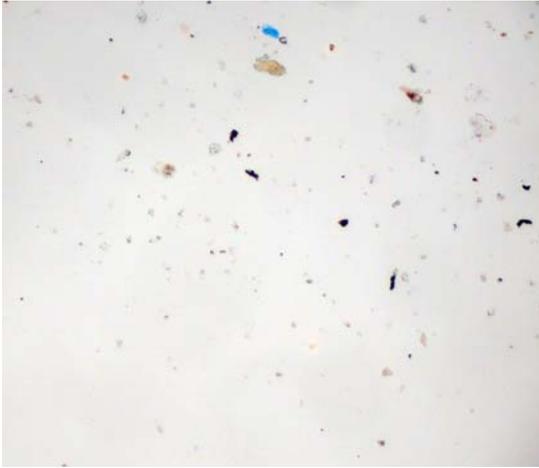


Figure 4. Example of a typical diffuse AOC sample trace at 100X magnification.

The corner of a typical BA sample trace at 100X magnification is illustrated in Figure 5. The edges of the sample trace were bounded by the compression ridges below the sample slit, and the boundary of the sample trace was sharply defined. The spores were retained within the shaded sample area with dimensions of 1.7 mm x 5.5 mm. The spores remained within the sample trace after transportation, handling and staining.

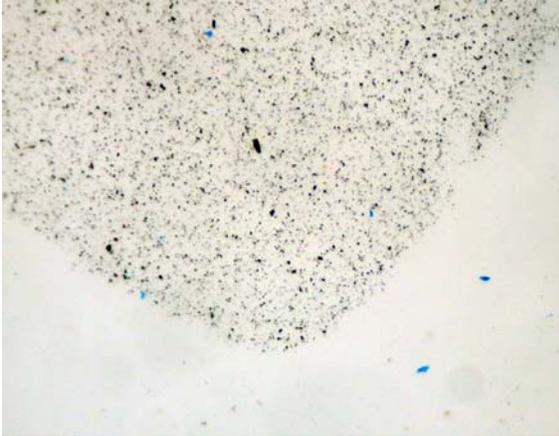


Figure 5. Example of a typical sharply defined BA sample trace at 100X magnification.

The MCE filters often became brittle if they were excessively dry, and the compression ridges frequently caused the filter to tear. However, the tear in the filter, as could be observed using either a stereoscope or at a magnification of 100x, occurred outside the compression ridge and did not affect the sample collection area nor the integrity of the sample.

### Filter Pore Size

The small exposed area of the MCE filter created a significant back pressure, limiting maximum airflow rates to about 3.7 lpm for a 1.2  $\mu\text{m}$  filter and 5.6 lpm for a 3  $\mu\text{m}$  filter when using a high volume air sampling pump. Airflow rates were further restricted when using a low volume personal sampling pump. Therefore, the effect of filter pore size was evaluated. The relative retention of *Asp/Pen* type and *Stachybotrys* spores for filters with pore sizes of 1.2, 3 and 5  $\mu\text{m}$  was evaluated for the BA. Since each BA produced two samples, the coefficient of variation (CV) in Table 1 was calculated using  $n = 4$  for each pore size. The percentage recoveries in Table 1 are relative to those obtained with a filter having a 1.2  $\mu\text{m}$  pore size.

Table 1. The relative retention of *Aspergillus/Penicillium* and *Stachybotrys* spores for 25 mm MCE filters with pore sizes of 1.2, 3 and 5  $\mu\text{m}$ .

PARAMETERS	<i>Asp/Pen</i>			<i>Stachybotrys</i>		
	1.2	3	5	1.2	3	5
Pore Size ( $\mu\text{m}$ )	1.2	3	5	1.2	3	5
Average (spores/ $\text{m}^3$ )	10,512	10,625	8,539	518	712	485
CV ( $n = 4$ )	9 %	13 %	21 %	20 %	29 %	52 %
Relative Concentration (1.2 $\mu\text{m}$ )	100 %	101 %	81 %	100 %	137 %	94 %

The average retention of *Asp/Pen* type spores was equivalent for filters with pore sizes of 1.2  $\mu\text{m}$  and 3  $\mu\text{m}$ . The use of filters with a 5  $\mu\text{m}$  pore size resulted in about a 20 % reduction in the average retention for the smaller *Asp/Pen* type spores. The variation in the retention rates for the larger *Stachybotrys* spores was not proportional to pore size. However, the CV was proportional to the pore size for both spore types.

### Airflow Rate

The effect of airflow rate on the aspiration and retention of spores was evaluated for the BA. The average spore concentrations obtained at airflow rates of 1, 2 and 3 lpm are contained in Table 2. In addition, the ratios of the BA concentrations to the AOC concentration were calculated for each airflow rate.

Table 2A. Average concentrations of *Aspergillus/Penicillium* and *Stachybotrys* spores (spores/ $\text{m}^3$ ) relative to the AOC concentration when the BA was tested at airflow rates of 1, 2 and 3 lpm.

AIRFLOW RATE	1 LPM	2 LPM	3 LPM
<i>Asp/Pen</i>			
AOC	9,080	10,344	10,487
BA	17,816	18,793	15,486
BA:AOC	2.0	1.8	1.5

Table 2B. Average concentrations of *Aspergillus/Penicillium* and *Stachybotrys* spores (spores/m<sup>3</sup>) relative to the AOC concentration when the BA was tested at airflow rates of 1, 2 and 3 lpm.

AIRFLOW RATE	1 LPM	2 LPM	3 LPM
<i>Stachybotrys</i>			
AOC	437	601	559
BA	595	571	546
BA:AOC	1.36	0.95	0.98

The performance of the BA was measured relative to that of the AOC at the three airflow rates, which remained under constant sampling conditions. The average ratios of AOC to BA concentrations for *Asp/Pen* spores were 2.0 and 1.8 at airflow rates of 1 lpm and 2 lpm, respectively. However, the ratio increased to 1.5 at an airflow rate of 3 lpm. The relative BA concentrations for *Asp/Pen* type spores decreased from 100 % at an airflow rate of 1 lpm, to 90 % at 2 lpm, to 75 % at 3 lpm. The ratios of BA to AOC concentrations for *Stachybotrys* varied between 1.36 and 0.95 for the three airflow rates, with an average of 1.08. A trend was not observed between the airflow rate used with the BA and the reported *Stachybotrys* concentration.

### Spore Deposition

The uniformity of spore deposition across the sample traces was compared for the AOC and BA. Table 3 contains the distribution of spores within the sample areas of the AOC and BA cassettes for six sample traces. The six samples were selected randomly, and the samples were considered to be representative for each sampler.

Table 3. Variation of spore counts between individual traverses for the AOC and BA cassettes at 600x magnification for six representative samples.

AIR-O-CELL CASSETTE		BI-AIR CASSETTE	
Spores / FOV	CV (%)	Spores / FOV	CV (%)
18	32	14	29
24	28	14	27
35	31	15	33
37	36	20	24
48	20	24	28
48	28	38	20

## Bi-Air Chamber Tests

The data in Table 3 are the average number of spores per FOV at 600X magnification in each sample, plus the average CV for the variation of spore counts between the traverses within each sample. The data are arranged by the average number of spores per field of view (FOV), which ranged from 18 to 48 for the AOC and from 14 to 38 for the smaller BA sample trace. The uniformity of the spores distributed over the sample areas was equivalent for the AOC and the BA, as indicated in Table 3. The average spore density at 600X magnification was 35 spores per FOV for the AOC, with an average CV of 29 %. The average spore density was 21 spores per FOV for the BA, with an average CV of 27 %.

## DISCUSSION

### Bi-Air Cassette

The BA had four inlets positioned on the side of the cowl at 90° to each other, as indicated in Figure 2. The variable-width inlets allowed the inlet velocity to be controlled while varying the airflow rate. In addition, the four inlets increased the isoaxial sampling properties of the sampler when oriented vertically. The aspirated sample in the BA entered an enclosed chamber formed by the cowl and cap; and was drawn through the two rectangular slits positioned directly above the filter. Tape lift samples were collected from the plastic strip separating the two sample slits in several samplers. No spores were detected, although the sample size was small.

Each inlet was a 3-mm square with an area of 0.09 cm<sup>2</sup>. Therefore, an airflow rate of 0.5 lpm resulted in an inlet velocity of 23 cm/sec. Therefore, the inlet velocity was less than 92 cm/sec for airflow rates of 2 lpm or less, typical of the range of air velocities detected in indoor spaces.<sup>(14)</sup> For airflow rates in the range of 0.5 - 3 lpm, inlet velocities would vary from 23 cm/sec to 138 cm/sec when the inlets were fully open.

The total exposed area of filter in the two sample slits illustrated in Figure 3 was 0.187 cm<sup>2</sup>. Therefore, at an airflow rate of 1 lpm, the face velocity at the filter surface would be expected to be 90 cm/sec. The face velocity was expected to vary from 45 cm/sec at an airflow rate of 0.5 lpm to 270 cm/sec at 3 lpm. Since particle penetration into the filter matrix was expected to decrease as the face velocity increased, the high face velocities in the BA were expected to help retain the spores near the surface of the filter; an advantage for analysis by microscopy.<sup>(15, 16)</sup>

Particle bounce may be an issue for both silt impaction samplers and filter samplers, especially at high filter face velocities.<sup>(15-20)</sup> However, when bounce occurs in an impaction sampler, the particle rebounds into a linear airflow that is moving parallel to the collection medium. This increases the probability that the particle will not be retained. In a filter sampler, the air flow is perpendicular to the collection medium. When bounce occurs, some of the kinetic energy of the particle is lost, and the air stream again directs the now lower-energy particle into repeated contact with the collection medium. Second, MCE filters have a rough surface, and the pores have a high degree of tortuosity, both of which tend to increase spore retention. Finally, once aspirated into the BA, the spore is contained in an enclosed chamber formed by the cassette cowl and cap. Therefore, sample loss in the BA due to particle bounce, even at high filter face

velocities, was expected to be of less importance relative to the loss incurred in slit impaction samplers.

The design of the BA, illustrated in Figure 2, is typical of a blunt sampler rather than a thin-wall sampler. This design was expected to be more efficient for the low air velocities encountered indoors, but less efficient for sampling in the presence of substantial wind velocities outdoors.<sup>(17)</sup> In addition, neither the Reynolds number, Stokes number, nor a requirement for isokinetic sampling were expected to be significant factors when sampling larger ( $\Rightarrow$  1  $\mu\text{m}$ ) particles in calm air.<sup>(17-20)</sup>

### **Bi-Air Characteristics**

The Bi-Air (BA) filter cassette, which was specifically designed for the collection of airborne fungal spores, had the following characteristics:

- Small size and ease of handling of a 25 mm filter cassette;
- Four side-inlets to increase the probability of isoaxial sampling;
- Width of the inlets may be varied with airflow rate to maintain a constant inlet velocity with variations in airflow rate;
- Low airflow rates of 0.5-3 lpm (or less), with the ability to vary the airflow rate while maintaining efficient sampling;
- Low inlet velocities of 20-138 cm/sec (and less) to minimize inlet turbulence;
- Low transmission losses based on a comparison of results obtained with the AOC;
- High filter face velocities of 45 to 270 cm/sec, which minimizes the penetration of even small spores into the filter matrix;
- Uniform particle deposition on the collection medium, equivalent to the AOC;
- Direct microscopic analysis without sample transfer;
- High collection efficiency of a filter medium;
- Exchangeable (MCE, gel, polycarbonate) 25 mm filter media;
- Ability to support a variety of analytical methods (microscopy, culturing, PCR);
- Applicable to a wide range of concentrations;
- Ability to collect both short-term (10 minute) and long-term(7 hour) samples;
- Constructed of carbon-filled polystyrene to minimize electrical effects.

Therefore, the BA had many of the characteristics that several authors have mentioned as being desirable for a bioaerosol sampler. In addition, it had the following three characteristics:

- a small (9.35 mm<sup>2</sup>) rectangular sample trace with well-defined boundaries;
- duplicate sample traces; and
- the ability to vary inlet size with airflow rate.

### Optimum Sampling Time

The optimal sampling time (T) for a sampler can be estimated for a moderately contaminated indoor environment using Equation 1.<sup>(21)</sup>

$$[1] \quad T = S A / C Q$$

The optimal sampling time is a function of the desired sample density (S), the sample area (A), the average analyte concentration (‘C), and the airflow rate (Q). The optimal sampling time was calculated for the BA assuming a concentration of 1,000 spores/m<sup>3</sup> and an optimum sample density of 10,000 spores/cm<sup>2</sup> for microscopy.<sup>(21)</sup> The optimal sampling time varied from 47 minutes at an airflow rate of 4 lpm to 376 minutes at an airflow rate of 0.5 lpm. The BA has typically been used in field investigations at an airflow rate of 1-2 lpm for periods of 60-90 minutes. A similar calculation for the AOC, assuming a sampling rate of 15 lpm and a typical deposit area of 29 mm<sup>2</sup>, indicates an optimum sampling time of 19 minutes.<sup>(2)</sup>

### Sample Trace

A standard 25 mm filter has an exposed filter area of 380 mm<sup>2</sup>, while in comparison the AOC cassette has a typical sample area of about 29 mm<sup>2</sup>.<sup>(2)</sup> The smaller area of the AOC allows the sample to be analyzed by direct microscopic examination. A primary advantage of the BA compared to other filter cassettes is that the sample is restricted to two small, rectangular areas on the filter, each with an area of 9.35 mm<sup>2</sup>. Each of the two sample traces is therefore about 1/3 the size of the sample trace typically produced by the AOC.<sup>(2)</sup> This feature eliminates one of the limitations of using filtration to collect and analyze airborne fungal spores, which is the large surface area of the filter.

Therefore, the size of the BA sample trace was sufficiently small to allow the direct microscopic examination of the sample without an intermediate sample transfer step. This allowed the entire sample area to be included in the analysis, and the results to be reported quantitatively. In addition, slit impaction samplers are constructed with a gap between the base of the inlet and the impaction plate. This gap causes the sample density to be bell-shaped, with greater density near the centerline of the inlet. This gap results in a sample trace with a diffuse boundary, as indicated in Figure 4. This is a photograph of one corner of an AOC sample trace at 100X magnification, indicating the poorly defined sample boundary. Since the inlet on a slit impaction sampler is approximately 1 mm x 14.5 mm, the area of the sample trace is typically at least twice this area, or 30 mm<sup>2</sup>.<sup>(2)</sup>

Figure 5 is a similar photograph of one corner of a BA sample trace at 100X magnification. The compression ridges along the bottom perimeter of each sample slit compressed the filter, retaining the entire sample within an area on the filter with dimensions of 1.7 mm x 5.5 mm. This limited the area that had to be searched by the analyst to detect the deposited spores.

The BA represented an effective method for utilizing the potential benefits offered by filter samplers for the collection of airborne fungal spores. The duplicate sample traces were small, well-defined, and easily analyzed by direct microscopic examination.

### **Spore Deposition**

The distribution of spores across the BA sample trace was compared to the distribution for the AOC, as indicated in Table 3. The density of spores in the comparison samples varied from 18 to 48 per FOV for the AOC, with a CV of 29 %. The density of spores for the BA in Table 3 varied from 14 to 38 per FOV, with a CV of 27 %. The variation of spore counts between traverses, as indicated in Table 3, was equivalent for the two samplers. In addition, the average magnitude of the variability between traverses (28 %) was considered to be reasonable for an environmental sample. Furthermore, a pattern in the spore concentration along the longitudinal axis of the sample trace was generally not detected for either sampler. Therefore, it was concluded that the spores were deposited with an equivalent uniformity by the two samplers.

### **Filter Pore Size**

The particle collection efficiency for filters was expected to be very high, even for particles significantly smaller than a stated filter pore size.<sup>(15, 19)</sup> The dominant spore collection mechanisms for filters with a pore size of 3  $\mu\text{m}$  are expected to be sieving and interception, since most spores have a diameter greater than 1  $\mu\text{m}$ . The importance of impaction as a collection mechanism was only expected to become significant for particles with a diameter of 1  $\mu\text{m}$  and smaller.<sup>(15, 19)</sup> The relative concentrations of *Asp/Pen* type spores in Table 1 indicated that the retention of these smaller spores was equivalent for filters with pore sizes of 1.2 and 3  $\mu\text{m}$ . However, a 20 % reduction in the reported concentration of *Asp/Pen* spores occurred for a filter pore size of 5  $\mu\text{m}$ . Therefore, an MCE filter with a 3  $\mu\text{m}$  pore size was the largest pore size that could be used without resulting in the loss of some of the small *Asp/Pen* type spores.

The maximum airflow rate that could be obtained through an MCE filter varied between filter lots, and individual filters within a lot, due to variations in the thickness of individual filters. The small exposed area of filter resulted in a substantial back pressure, limiting the airflow rate. For example, maximum airflow rates for MCE filters with a pore size of 1.2  $\mu\text{m}$  varied from 2.8 lpm to 4 lpm, with a typical maximum airflow rate of 3.7 lpm. Therefore, the airflow rate should be calibrated for each cassette.

### **Airflow Rate**

The collection efficiency of a filter sampler for typical fungal spores is not limited by cut size or spore morphology.<sup>(2, 5, 15, 17-20)</sup> Therefore, it was expected that the retention of the smaller *Asp/Pen* type spores by the BA would be higher relative to the AOC slit impaction sampler. If the BA were an efficient sampler, the concentration of *Asp/Pen* type spores detected with the AOC in side-by-side samples were expected to be about half that detected with the BA.<sup>(2, 5)</sup>

Therefore, the ratio of BA to AOC concentrations was expected to be about 2.0 for the *Asp/Pen* type spores.

Table 2 contains concentrations of *Asp/Pen* and *Stachybotrys* spores collected in side-by-side BA samples at airflow rates of 1 lpm, 2 lpm, and 3 lpm. The actual ratios of BA to AOC concentrations for *Asp/Pen* were 2.0 and 1.8 at airflow rates of 1 and 2 lpm, respectively. These results suggested the BA was an efficient sampler for small spores at low airflow rates. However, the ratio for *Asp/Pen* was 1.5 at an airflow rate of 3 lpm, a 25 % reduction in reported concentration compared to an airflow rate of 1 lpm. This result indicated a reduced retention of spores by the BA at the higher airflow rate, and suggested that turbulence may have developed at the inlets when the inlet velocity exceeded approximately 92 cm/sec, which was equivalent to an airflow rate of 2 lpm with fully open inlets.

Based on the observed ratios, it was estimated that the BA concentrations of smaller spores may be under reported by approximately 20 % at an airflow rate of 3 lpm. However, even when sampling at 3 lpm, the concentration of *Asp/Pen* type spores expected to be reported with the BA would still be 50 % higher than with the AOC. The ratios for the larger *Stachybotrys* spores collected with the BA, contained in Table 2, varied from 1.36 to 0.95 of the AOC concentration. The average BA concentration was 108 % of the average AOC concentration. Therefore, the aspiration efficiency of the BA did not decrease substantially for airflow rates of 1 to 3 lpm when collecting the larger spores. Although absolute concentrations in the settling chamber were not measured, the relative retention efficiencies of the two samplers suggested the BA was an efficient sampler for fungal spores collected under calm-air conditions. The collection of the smaller *Asp/Pen* type spores was efficient at airflow rates up to 2 lpm; and the collection of the larger *Stachybotrys* spores was efficient at airflow rates up to 3 lpm.

### **Limitations**

The comparative study described in this article had several limitations. First, the actual spore concentrations in the settling chamber were not determined. Therefore, the performance of the AOC and the BA could only be compared on a relative basis, and the experimental design was limited to a comparison of the two samplers using side-by-side sampling.

In addition, the study was based on a relatively small number of samples that were collected with each sampler. Although lacking in statistical power, the results were sufficiently consistent to suggest that the relative performance of the two samplers was repeatable.

Third, only a limited range of concentrations were evaluated. The maximum concentration of *Asp/Pen* type spores was 30,000 spores/m<sup>3</sup>; and the maximum *Stachybotrys* concentration was 1,200 spores/m<sup>3</sup>. Therefore, the results of the study may not apply at substantially higher concentrations. However, the concentration ranges included in the study were typical of those often encountered in field studies.

Finally, the comparison was limited to only two spore types, a smaller *Asp/Pen* type spore and the larger *Stachybotrys* spore. However, *Asp/Pen* type spores are often the dominant spore type detected in contaminated indoor environments. Therefore, the relative performance of the two samplers demonstrated in this study were expected to be reflected in the collection of indoor samples.

## SUMMARY

A filter sampler has been described that allows the sample to be analyzed by direct microscopic examination without an intermediate sample transfer step. In addition, it produces duplicate sample traces. Each BA sample trace was confined to an area of 9.35 mm<sup>2</sup>, with a total sample area of 18.7 mm<sup>2</sup> for the duplicate sample traces. This area was about 67 % of the typical AOC sample trace. The duplicate sample traces produced by the BA were bounded and well defined, while the sample trace produced by the AOC was diffuse with poorly defined boundaries

The ratio of the BA concentration to the AOC concentration for *Asp/Pen* type spores was 2.0 at an airflow rate of 1 lpm, 1.8 at an airflow rate of 2 lpm, and 1.5 at an airflow rate of 3 lpm. The BA was less efficient at the higher airflow rate of 3 lpm. The retention of the larger *Stachybotrys* spores by the AOC was about equal to that of the BA at all three airflow rates.

MCE filters with pore sizes of 1.2 um and 3 um had an equivalent spore retention for 3 um *Asp/Pen* type spores. A pore size of 5 um resulted in a 20 % reduction in the measured concentration of *Asp/Pen* type spores, but not the larger *Stachybotrys* spores.

## REFERENCES

1. Macher, J.M. (1997) "Evaluation of bioaerosol sampler performance", *Appl. Occup. Environ. Hyg.*, 12, 730-36.
2. Aizenberg, V., Reponen, T., Grinshpun, S.A., Willeke, K. (2000) "Performance of Air-O-Cell, Burkard and Button Samplers for Total Enumeration of Airborne Spores, *AIHAJ*, 61, 855-864.
3. Chen, B.T., Feather, G.A., Maynard, A, Rao, C.Y. (2004) "Development of a Personal Sampler for Collecting Fungal Spores", *Aerosol Sci & Tech*, 38, 926-37.
4. Baxter, D.M., Perkins, J.L., McGhee, C.R., Seltzer, J.M (2005) "A Regional Comparison of Mold Spore Concentrations Outdoors and Inside "Clean" and "Mold Contaminated" Southern California Buildings", *JOEH*, 2, 8-18.
5. Trakumas, S., Willeke, K., Reponen, T., Trunov. M.(1998) "Particle Cut-Size Evaluation of Air-O-Cell Sampler", Report:3-27-98, Zefon International, St. Petersburg, FL.

## Bi-Air Chamber Tests

6. Palmgren, U., Strom, G., Blomquist, G., Malmberg, P. (1986) "Collection of airborne microorganisms on Nucleopore Filters, estimation and analysis - CAMNEA method", *J. Appl. Bacteriol.*, 61, 401-406.
7. Eduard, W., Lacey, J., Karlsson, K., Palmgren, U., Strom, G., Blomquist, G. (1990) "Evaluation of methods for enumerating microorganisms in filter samples from highly contaminated occupational environments", *AIHAJ*, 51,427-436.
8. Aizenberg, V., Grinshpun, S.A., Willeke, K., Smith, J., Baron, P.A. (2000) "Performance Characteristics of the Button Personal Inhalable Aerosol Sampler", *AIHAJ*, 61, 398-404.
9. McGrath, J.J., Wong, W.C., Cooley, J.D., Straus, D.C. (1999) "Continually measured fungal profiles in sick building syndrome", *Curr. Microbiol.*, 38(1), 33-6.
10. Engelhart, S., Exner, M., (2002) "Short-term versus long-term filter cassette sampling for viable fungi in indoor air: comparative performance of the Sartorius MD 80 and GSP sampler", *Int. J. Hyg. Environ. Health*, 205(6), 443-51.
11. NIOSH Method 7400: Asbestos and Other Fibers by PCM; NIOSH Manual of Analytical Methods, Fourth Edition, 8/15/94.
12. Bi-Air Corp, Placentia, CA 92870.
13. (2006) "A low cost settling chamber for comparing bioaerosol samplers", *Indoor Air* [Pending].
14. Baldwin, P.E.J., Maynard, A.D. (1998) "A Survey of Wind Speeds in Indoor Workplaces", *Ann. Occup. Hyg.*, 42(5), 303-13.
15. Brock, T.D. (1983) *Membrane Filtration: A User's Guide and Reference Manual*; Science Tech, Inc., Madison, WI.
16. Hering, S.V., Tech. Ed. (1989) *Air Sampling Instruments for evaluation of atmospheric contaminants*, 7<sup>th</sup> ed.; ACGIH, Cincinnati, OH.
17. Vincent, J.H., Ed. (1999) *Particle Size-Selective Sampling for Particulate Air Contaminants*; ACGIH, Cincinnati, OH.
18. Willeke, K., Baron, P.A., Ed. (1993) *Aerosol Measurement: Principles Techniques and Applications*; Van Nostrand Reinhold, NY, NY.
19. Hinds, W.C. (1999) *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles*; 2<sup>nd</sup> Ed.; John Wiley & Sons, NY, NY.

## Bi-Air Chamber Tests

20. Vincent, J. H. (1995) *Aerosol Science for Industrial Hygienists*; Pergamon, Elsevier Science, Tarrytown, NY.
21. Macher, J., Ed. (1999) *Bioaerosols: Assessment and Control*; ACGIH, Cincinnati, OH.
22. Schulster L M, et al. (2004) “Guidelines for Environmental Infection Control in Health-Care Facilities: Recommendations from CDC and the Healthcare Infection Control Practices Advisory Committee” (HICPAC), ASHE/AHA, Chicago, IL.