

Estimation of airborne fungal flora by the Andersen sampler versus the gravity settling culture plate

I. Isolation frequency and numbers of colonies

William J. Sayer, M.D., Dudley B. Shean, Ph.D., and
Jamshid Ghossein, B.S., Menlo Park, Calif.

The gravity settling culture (GSC) plate and the sequential impaction cascade sieve volumetric air (SICSV) sampler devised by Andersen were critically compared in the estimation of airborne fungal flora. One hundred twenty-seven side-by-side simultaneous 15 minute samples were obtained on Mehrlich's medium in Northern California. The SICSV sampler collected 25 fungal types totaling 14,300 colonies, and the adjacent GSC plates displayed 18 genera in 1,091 colonies. Statistically significant differences in total colonies and frequency of isolation were noted for Penicillium, Alternaria, Aspergillus, Gliocladium, Pullularia, Rhizopus, Saccharomyces, Stemphylium, Ustilago zeae, and other (unidentified) fungi. The capability of the SICSV sampler to isolate volumetrically in a reproducible fashion numbers of airborne fungal spores was demonstrated. It is suggested that these colonies can be logically grouped into a fraction that generally would lodge in the upper respiratory tract and another fraction which, because of the small spore sizes, would largely be capable of lung penetrance. Both methods demonstrated a capacity to produce falsely negative data, but this was especially true for the GSC plate. The GSC plate method cannot be endorsed for the quantitative evaluation of airborne fungal spores.

Estimations of the types and numbers of fungi in the air may serve several useful purposes: comparison of climates or microclimates¹; evaluation of environmental air hygiene measures²; correlation of the mold spores in the respired air with symptoms, allergy skin tests, or the prescribed hyposensitization programs.³ All of these purposes would best be served by use of the most efficient or accurate sampling technique.

From the Stanford University School of Medicine, and the Santa Clara Valley Medical Center.

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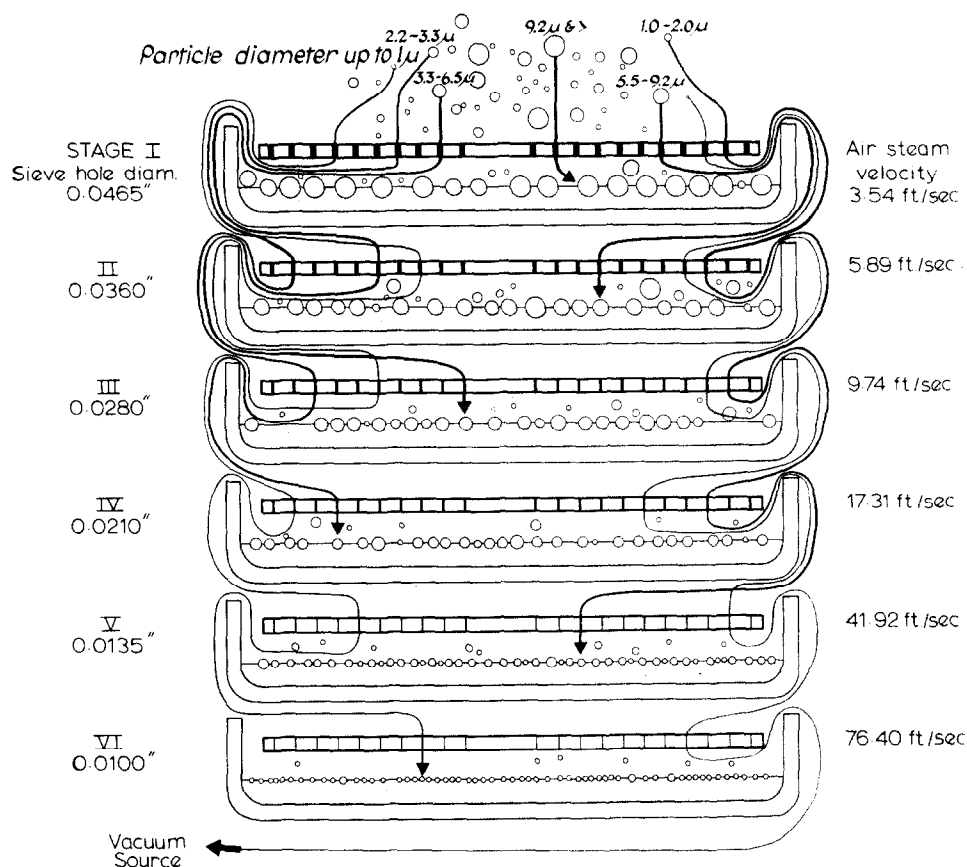


Fig. 1

Cross-sectional schematic diagram of an Andersen or sequential impaction cascade sieve volumetric air (SICSVA) sampler. Note the decreasing sieve-hole size with increasing air-stream velocity from top to bottom. Idealized particle spheres are greatly magnified to illustrate the collection of particles and the classification by sizes on the media surfaces of the six stages. See Materials and methods.

Though simple and inexpensive, the gravity settling culture (GSC) plate can scarcely be described as accurate. It is not recommended for the quantitative evaluation of airborne flora⁴ because it collects only those particles large enough to be pulled by gravity or impacted by air turbulence onto the collecting surface. Thus, airborne particles that are too small to settle out quickly may not be collected, and if the turbulence of the air to be sampled varies from time to time, the yields of the GSC plate will also vary. The long exposures needed to counterbalance these factors may result in drying of the media with consequent poor growth.⁵

Andersen's sequential impaction cascade sieve volumetric air (SICSVA) sampler⁶ (Fig. 1) efficiently collects both large and small airborne particles and classifies these according to size. This sampler is highly recommended.^{4, 6, 7} Indeed, Cole and associates⁵ found it to be at least 5 times as efficient as the

GSC plate, in $\frac{1}{6}$ of the sampling time, while collecting coagulase-positive staphylococci from hospital operating-room air.

The SICSVA sampler has been used to isolate thermophilic actinomyces artificially suspended in the air by agitation of hay associated with cases of "farmer's lung."⁹ It has proven superior to the all-glass liquid impinger in isolating *Coccidioides immitis* from the dusty air of Camp Roberts, California.¹⁰

The following study was done on the premise that a critical, side-by-side comparison of the GSC plate and the SICSVA sampler should yield information sufficient to support, deny, or amplify these recommendations.

MATERIALS AND METHODS

Each sterile GSC plate, a conventional 100 x 10 mm. glass culture dish, received 15 ml. of sterile Mehrlich's agar (Difco No. 0814) at about 45° C. Each of the SICSVA sampler's sterile, specially designed 90 x 16 mm. thick-bottomed glass dishes received 27 ml. of the same medium from a Manostat adjustable-volume syringe. After cooling to room temperature, all culture plates were refrigerated at 5° C. until just prior to use. Care was taken to avoid the drying effects of long exposures to room temperature or direct sunlight.

Before each use, the SICSVA sampler was cleaned and steam autoclaved for 15 minutes at 121° C. with 15 pounds of pressure.

The vacuum pump for the SICSVA sampler was always warmed for 20 minutes prior to the sampling to assure a constant vacuum source. Hansen quick-disconnect couplings with $\frac{3}{8}$ inch (inside diameter) vacuum tubing were used in order to reduce the variations that can be produced by hose kinking or loose connections. An electric timer switch with visual and audible alarms and automatic current interruption provided accurate timing. The vacuum pump was flow rated before each sample with a Fischer Porter Flow-meter Model A-60 in order to insure a sampling rate of one cubic foot (28.3 L.) per minute, the usual flow rate at which this sampler is designed to operate.

Simultaneous, paired, side-by-side GSC plate and SICSVA samples of 15 minutes' duration were obtained. A sampling height of 30 inches above the floor was maintained in hospital rooms and operating suites, but 60 inches was the sampling height elsewhere.

The GSC plate was uncovered when the timer switch activated the warmed vacuum pump for the adjacent SICSVA sampler. Fifteen minutes later, when the timer switch alarm sounded and the vacuum pump was shut off, the cover was replaced on the GSC plate, and the SICSVA sampler was disconnected, unloaded, and disassembled.

Following exposure, the GSC and the SICSVA sampling plates were covered, inverted, and incubated at 26 to 32° C. for 5 to 10 days. Care was taken not to move the plates during that time because our earlier, unreported studies showed secondary or daughter colonies in 5-day-old plates which had been moved or agitated after 24 hours of incubation. Uniform colony-count conditions were provided by the use of the Acculite illuminated magnifying reader, Scientific Products No. B 1078.

All colonies found in Stage 6 were considered to be contaminants for the following reasons: These colonies were all located in places on the media sur-

face other than the usual impaction pattern. The aerodynamics of the sampler as designed⁶ and critically evaluated¹² suggest that almost all particles penetrating to this stage are of less than 1 μ in diameter, much smaller than the usual airborne fungal spore. There were ample opportunities for contamination to occur during medium preparation and loading and unloading of the SICSVA sampler. Since these opportunities were present for the GSC plates and the SICSVA sampler plates alike, the few colonies found in Stage 6 (Chart IV) were not excluded from the statistical totals of colonies when the 2 sampling methods were compared.

Identification of individual fungal type was made from several colony characteristics, including colony growth, gross appearance of colony surface and margin, color, odor, and color of undersurface, and microscopic examination with D'Antoni's iodine stain.

Data were processed on an IBM 360/67 computer at the Stanford University Computation Center. Chi square tests for equality of proportions were performed on the grouped data and for each fungal type separately.

RESULTS

We obtained 130 paired tests. A paired test consisted of simultaneous air sampling by a GSC plate and a SICSVA sampler, side-by-side, for 15 minutes. Because of gross fungal contamination of both the GSC and SICSVA sampler

Chart I. Total airborne fungal yields of the SICSVA (Andersen) sampler compared to the accompanying gravity settling culture (GSC) plate (127 paired tests)

<i>Fungus</i>	<i>SICSVA* sampler (Andersen) total colonies</i>	<i>GSC plate total colonies</i>
Hormodendrum sp.	7,515 (52.6%)	551 (50.5%)
Penicillium sp.	3,000 (20.9%)	95 (8.8%)
Aspergillus sp.	1,310 (9.2%)	60 (5.5%)
Saccharomyces sp.	603 (4.3%)	167 (15.4%)
Other fungi (unidentified)	556 (3.9%)	51 (4.7%)
Gliocladium sp.	276 (1.9%)	11 (1.0%)
Alternaria sp.	195 (1.4%)	26 (2.3%)
Stemphilium sp.	178 (1.3%)	26 (2.3%)
Pullularia sp.	143 (1.0%)	10 (0.9%)
Epicoecum sp.	125 (0.8%)	58 (5.4%)
Monotospora sp.	109 (0.7%)	15 (1.4%)
Hemispora sp.	81	4
Rhizopus sp.	60	1
Ustilago zaeae	50	0
Diplospora sp.	23	1
Paecilomyces sp.	23	4
Sepedonium sp.	12	0
Oospora sp.	10 (2.0%)	7
Geotrichum sp.	9	3 (1.8%)
Rhodotorula sp.	9	0
Candida albicans	5	1
Streptomyces sp.	4	0
Nonsporulative unidentified fungi	2	0
Monilia sitophila	1	0
Nigrospora sp.	1	0
Totals	14,300 (100%)	1,091 (100%)

*Sequential impaction cascade sieve volumetric air sampler.

plates most likely incurred during medium preparation and plate pouring, 3 paired tests were discarded.

In the 127 valid comparisons, the GSC plates showed only 1,091 fungal colonies, while the SICSVA sampler plates displayed 14,300 (Chart I). Hormodendrium, Penicillium, Aspergillus, and Saccharomyces accounted for most of the colonies in both sampling methods, though in somewhat different order (Chart I).

The 2 methods differed in total colonies obtained by species; the differences were statistically highly significant for Penicillium, (p value less than 0.001); significant for Alternaria, Aspergillus, Gliocladium, Hemispora, Hormodendrium, Monotospora, Pullularia, Rhizopus, Saccharomyces, Stemphilium, *Ustilago zeae*, and other fungi ($p < 0.01$); of borderline significance for Diplospora, Epicoccum, Paecilomyces, Rhodotorula, Sepedonium, and Streptomyces; and could have happened by chance for *Candida albicans*, *Monilia sitophila*, Geotrichum, Nigrospora, Oospora, and nonsporulating unidentified fungi.

The number of fungal colonies per GSC plate ranged from 0 to 48 with

Chart II. Percentage of positive isolations of the SICSVA (Andersen) sampler compared to the accompanying gravity settling culture (GSC) plates (127 paired tests)

Fungus	SICSVA* Sampler (Andersen) positive isolations	Per cent of 127 tests	GSC plate positive isolations	Per cent of 127 tests
Penicillium sp.	127	100	50	39
Hormodendrum sp.	125	98	96	76
Aspergillus sp.	118	93	31	24
Saccharomyces sp.	102	80	59	46
Other fungi (unidentified)	83	65	27	21
Stemphilium sp.	74	58	24	19
Alternaria sp.	53	42	19	15
Hemispora sp.	51	40	3	2
Epicoccum sp.	49	39	27	21
Gliocladium sp.	44	35	5	4
Monotospora sp.	36	28	11	8
Rhizopus sp.	29	23	1	0.8
<i>Ustilago zeae</i>	27	21	0	0
Pullularia sp.	26	20	4	3
Paecilomyces sp.	7	6	2	1.5
Diplospora sp.	7	6	1	0.8
<i>Candida albicans</i>	4	3	1	0.8
Geotrichum sp.	4	3	3	2
Oospora sp.	4	3	5	4
Sepedonium sp.	4	3	0	0
Rhodotorula sp.	3	2	0	0
Streptomyces sp.	3	2	0	0
<i>Monilia sitophila</i>	1	0.8	0	0
Nigrospora sp.	1	0.8	0	0
Nonsporulating unidentified fungi	1	0.8	0	0
Totals	983		369	

*Sequential impaction cascade sieve volumetric air sampler.

an average of 8.6; the number of colonies per set of SICSVA plates ranged from 5 to 283, with an average of 112.7.

Eight GSC plates displayed no fungal growth, whereas the accompanying 8 sets of SICSVA sampler plates exhibited from 5 to 91 colonies of from 2 to 8 fungal types per set, with an average of 37 colonies of 6 genera and a collective total of 296 colonies. On one of these 8 occasions, there were 8 different fungal types (91 colonies) found by the SICSVA sampler including *Alternaria*, *Aspergillus*, *Hormodendrum*, *Penicillium*, *Rhizopus*, *Saccharomyces*, *Hemiaspora*, and other unidentified fungi. None of the SICSVA samples was negative.

Eighteen different fungal types were collected by the GSC plates in contrast to the 25 different types isolated by the SICSVA sampler ($p < 0.01$). The number of different genera collected by a single GSC plate ranged from 0 to 5, with an average of 2.9, but ranged from 2 to 12 for the SICSVA sampler, with an average of 7.7.

The same 11 fungal types accounted for 98 per cent of the SICSVA sampler colonies and 98.2 per cent of the GSC plate colonies. The 7 categories of fungi isolated by the Andersen sampler but not the GSC plate comprised only about half of one per cent of the total number of SICSVA sampler colonies. The same 4 genera that accounted for the largest numbers of fungal colonies were most frequently isolated by both methods (Chart II).

There were, however, appreciable differences in the percentage of positive isolations when the 2 methods were compared. The top 4 genera were absent 0, 2, 7, and 20 per cent of the time when the SICSVA sampler was used but absent as evidenced by the GSC plate for 61, 24, 76, and 54 per cent of the tests. The differences illustrated in detail in Chart II were so great that statistical analysis was not employed.

There appeared to be differences in colony counts originating from large versus smaller-sized spores. The various fungi were listed in decreasing order

Table I

Spore size classification	Total colonies	
	GSC plate	SICSVA sampler
Large spore size		
<i>Alternaria</i> , <i>Stemphilium</i> , <i>Epicoccum</i> , <i>Nigrospora</i> , <i>Diplospora</i> , <i>Monotospora</i> , and <i>Sepe-donium</i> sp.	126	643
Intermediate spore size		
<i>M. sitophila</i> , <i>Geotrichum</i> , <i>C. albicans</i> , <i>Pullu-laria</i> , <i>Saccharomyces</i> , <i>Aspergillus</i> , <i>Hormo-dendrum</i> , and <i>Penicillium</i> sp.	887	12,586
Small spore size		
<i>Ustilago zaeae</i> , <i>Rhodotorula</i> , <i>Rhizopus</i> , <i>Oospora</i> , <i>Gliocladium</i> , <i>Paecilomyces</i> , <i>Hemi-sporea</i> , and <i>Streptomyces</i> sp.	27	513
Totals	1,040	13,742

These differences are highly significant: $p < 0.001$.

Chart III. Airborne fungal yields of the gravity settling culture (GSC) plate compared to the accompanying SICSVA (Andersen) sampler listed in decreasing magnitude of approximate spore size (127 paired tests)

GSC plate colonies	Fungus	Spore size (microns)	SICSVA* sampler colonies
<i>Large spore sizes</i>			
26	<i>Alternaria</i> sp.	10-15 × 20-90	195
26	<i>Stemphilium</i> sp.	12-20 × 25-40	178
58	<i>Epicoccum</i> sp.	20 × 25	125
0	<i>Nigrospora</i> sp.	11 × 14	1
1	<i>Diplospora</i> sp.	2.3-17 × 10-18	23
15	<i>Monotospora</i> sp.	6.5-9 × 9.5-16	109
0	<i>Sepedonium</i> sp.	2-12 × 3-20	12
126			643
<i>Intermediate spore sizes</i>			
0	<i>Monilia sitophila</i>	5.2 × 13.4	1
3	<i>Geotrichum</i> sp.	5-10 × 4-5	9
1	<i>Candida albicans</i>	2-8 × 4-20	5
10	<i>Pullularia</i> sp.	4-7 × 8-21	143
167	<i>Saccharomyces</i> sp.	1-6 × 1-20	603
60	<i>Aspergillus</i> sp.	2-7 × 2.5-10	1,310
551	<i>Hormodendrum</i> sp.	2-4 × 8-12	7,515
95	<i>Penicillium</i> sp.	2-7 × 2.5-10	3,000
887			12,586
<i>Small pore sizes</i>			
0	<i>Ustilago zeae</i>	2.5 × 3-11	50
0	<i>Rhodotorula</i> sp.	2.5 × 8	9
1	<i>Rhizopus</i> sp.	3 × 6	60
7	<i>Oospora</i> sp.	2-5 × 8-18	10
11	<i>Gliocladium</i> sp.	2.5-3 × 4-7	276
4	<i>Paecilomyces</i> sp.	2-4 × 3-6	23
4	<i>Hemispora</i> sp.	2 × 5	81
0	<i>Streptomyces</i> sp.	0.8 × 0.8-7.5	4
27			513
Subtotals			
1,040			13,742
<i>Unknown spore sizes</i>			
0	Nonsporulative unidentified fungi		2
51	Other fungi (unidentified)		556
51			558
Totals			
1,091			14,300

*Sequential impactation cascade sieve volumetric air sampler.

of approximate spore size and arbitrarily separated into 3 groups: large, intermediate, and small spore sizes. The spore size could not be determined for the unidentified colonies listed as "other fungi" and the "nonsporulative unidentified fungi." Accordingly, these 2 categories were listed separately from the arbitrary groupings in Charts III, IV, V, and VI and Tables II and III but omitted from Table I, which illustrates the highly significant differences between the 2 methods in relation to approximate spore size.

Chart IV. Airborne fungal colony yields as classified by the individual six stages of the SICSVA (Andersen) sampler (listed in decreasing magnitude of approximate spore size)

SICSVA* sampler colonies	Fungus	Spore size (microns)	SICSVA* sampler stages					
			1	2	3	4	5	6
Large spore sizes								
195	Alternaria	10-15 × 20-90	130	56	8	1	0	0
178	Stemphilium	12-20 × 25-40	138	32	7	1	0	0
125	Epicoecum	20 × 25	115	10	0	0	0	0
1	Nigrospora	11 × 24	1	0	0	0	0	0
23	Diplospora	2.3-17 × 10-18	10	10	1	2	0	0
109	Monotospora	6.5-9 × 9.5-16	47	58	4	0	0	0
12	Sepedonium	2-12 × 3-20	6	3	0	3	0	0
Intermediate spore sizes								
1	Monilia sitophilia	5.2 × 13.4	0	1	0	0	0	0
9	Geotrichum	5-10 × 4-5	9	1	0	0	0	0
5	Candida albicans	2-8 × 4-20	1	2	1	0	1	0
143	Pullularia	4-7 × 8-21	33	31	39	40	0	0
603	Saccharomyces	1-6 × 1-20	341	113	62	33	53	1
1,310	Aspergillus	2-7 × 2.5-10	142	272	566	323	5	2
7,515	Hormodendrum	2-4 × 8-12	2,195	2,814	2,070	420	9	7
3,000	Penicillium	2-7 × 2.5-10	219	226	947	1,478	121	9
Small spore sizes								
50	Ustilago zeae	2-5 × 3-11	0	2	0	6	42	0
9	Rhodotorula	2.5-8	2	2	3	2	0	0
60	Rhizopus	3 × 6	7	26	25	1	0	1
10	Oospora	2-5 × 8-18	0	0	6	0	4	0
276	Gliocladium	2.5-3 × 4-7	14	9	48	165	40	0
23	Paecilomyces	2-4 × 3-6	0	15	6	2	0	0
81	Hemispora	2 × 5	4	5	9	38	24	1
4	Streptomyces	0.8 × 0.8-7	1	2	0	0	0	1
Unknown spore sizes								
2	Nonsporulative unidentified fungi		2	0	0	0	0	0
556	Other fungi (unidentified)		187	148	105	84	31	1
Totals								
14,300			3,604	3,837	3,907	2,599	330	23

*Sequential impaction cascade sieve volumetric air sampler.

Since the SICSVA sampler is said to classify the collected particles with respect to size, we have, based upon a review of the subject by Davies,¹¹ arbitrarily defined the fractional yields of the Andersen sampler as either non-lung penetrating* or lung penetrating.†

If the GSC plate collects largely heavy particles and should there be some

*Non-lung penetrating: the "catch" or number of colonies on Stages 1 and 2, which mainly originate from spores large enough (if inspired) to lodge in the upper respiratory tract of man, i.e., the nose, pharynx, larynx, trachea, and larger bronchi above the lung parenchyma.

†Lung penetrating: the "catch" or number of colonies on Stages 3, 4, 5, and 6, which mainly originate from spores small enough (if inspired) to penetrate deeply into the lower respiratory tract of man, the smaller bronchi, and bronchioles within the lung parenchyma.

Table II

<i>Spore size classification</i>	<i>Total colonies</i>	
	<i>GSC plate</i>	<i>Non-lung-penetrating fraction SICSVA</i>
Large	126	616
Intermediate	887	6,399
Small	27	89
Totals	1,040	7,104

Table III

<i>Spore size classification</i>	<i>Number of colonies of known identity</i>	
	<i>Non-lung-penetrating fraction</i>	<i>Lung-penetrating fraction</i>
Large	616	27
Intermediate	6,399	6,187
Small	89	424
Totals	7,104	6,638

validity to the premise that Stages 1 and 2 of the Andersen sampler (Chart IV) collect mainly those particles greater than $5\ \mu$ of mass median diameter (the non-lung-penetrating fraction*), a comparison of these should be of interest (Table II).

The GSC plate, like Stages 1 plus 2 of the SICSVA sampler, collects small and intermediate as well as the large size spores. The non-lung-penetrating yield of the Andersen sampler is considerably more efficient in all size ranges than the GSC plate, $p < 0.01$.

Data on spore size as related to collection by lung-penetrating and non-lung-penetrating portions of the SICSVA sampler are condensed from Chart IV and listed in Table III.

Clearly, most of the largest fungal spores are collected in the non-lung-penetrating portion of the SICSVA sampler, and most of the particularly small spores find a way to the lung-penetrating region ($p < 0.001$), while intermediate size spores are trapped almost equally in both areas (Chart IV, Table III).

Some fungi not isolated by a specific GSC plate were isolated by the accompanying SICSVA sampler in every one of the 127 tests. In these instances, single GSC plates were negative and the SICSVA sample positive for from one to 10 different genera or species, averaging 5.2 per sampling pair. There were 660 type misses for the GSC plates, totaling 4,374 colonies, for an average of

*Non-lung penetrating: the "catch" or number of colonies on Stages 1 and 2, which mainly originate from spores large enough (if inspired) to lodge in the upper respiratory tract of man, i.e., the nose, pharynx, larynx, trachea, and larger bronchi above the lung parenchyma.

Chart V. Instances of negative gravity settling culture (GSC) plates when the accompanying SICSVA (Andersen) sampler was positive for specific airborne fungi (127 paired tests)

Number of test pairs	Fungus	Spore size (microns)	Number of GSC plate colonies	Number of SICSVA* sampler colonies
Large spore sizes				
42	<i>Alternaria</i> sp.	10-15 × 20-90	0	160
57	<i>Stemphilium</i> sp.	12-20 × 25-40	0	132
33	<i>Epicoceum</i> sp.	20 × 25	0	79
1	<i>Nigrospora</i> sp.	11 × 14	0	1
6	<i>Diplospora</i> sp.	2.3-17 × 10-18	0	22
26	<i>Monotospora</i> sp.	6.5-9 × 9.5-16	0	81
4	<i>Sepedonium</i> sp.	2-12 × 3-20	0	12
169			0	487
Intermediate spore sizes				
1	<i>Monilia sitophilia</i>	5.2 × 13.4	0	1
4	<i>Geotrichum</i> sp.	5-10 × 4-5	0	9
3	<i>Candida albicans</i>	2-8 × 4-20	0	4
23	<i>Pullularia</i> sp.	4-7 × 8-21	0	118
50	<i>Saccharomyces</i> sp.	1-6 × 1-20	0	202
87	<i>Aspergillus</i> sp.	2-7 × 2.5-10	0	747
29	<i>Hormodendrum</i> sp.	2-4 × 8-12	0	575
77	<i>Penicillium</i> sp.	2-7 × 2.5-10	0	1,475
274			0	3,131
Small spore sizes				
27	<i>Ustilago zeae</i>	2-5 × 3-11	0	50
3	<i>Rhodotorula</i> sp.	2.5 × 8	0	9
28	<i>Rhizopus</i> sp.	3 × 6	0	58
2	<i>Oospora</i> sp.	2-5 × 8-18	0	5
39	<i>Cliocladium</i> sp.	2.5-3 × 4-7	0	219
5	<i>Paecilomyces</i> sp.	2-4 × 3-6	0	16
50	<i>Hemispora</i> sp.	2 × 5	0	79
3	<i>Streptomyces</i> sp.	0.8 × 0.8-7.5	0	4
157			0	440
Subtotals				
600			0	4,058
Unknown spore sizes				
1	Nonsporulative unidentified fungus (no spores formed)		0	2
59	Other fungi (unidentified)		0	314
Totals				
660			0	4,374

*Sequential impaction cascade sieve volumetric air sampler.

34.3 colonies per sampling experience (Chart V).

Separate fungal types not detected by the SICSVA sampler but present in the accompanying GSC plate were noted 46 times in 41 of the 127 test pairs. The number of types not collected by an individual SICSVA sampler but present in the corresponding GSC plate ranged from 0 to 2 per sample (average 1.1), a total of 67 colonies (Chart VI).

Chart VI. Instances of negative SICSVA (Andersen) sampler yields when the accompanying gravity settling culture (GSC) plate was positive for specific airborne fungi (127 paired tests)

Number of test pairs	Fungus	Spore size (microns)	Number of GSC plate colonies	Number of SICSVA* sampler colonies
<i>Large spore sizes</i>				
8	Alternaria sp.	10-15 × 20-90	13	0
7	Stemphilium sp.	12-20 × 25-40	7	0
11	Epicoccum sp.	20 × 25	20	0
<u>1</u>	Monotospora sp.	6.5-9 × 9.5-16	<u>1</u>	<u>0</u>
27			41	0
<i>Intermediate spore sizes</i>				
3	Geotrichum sp.	5-10 × 4-5	3	0
1	Pullularia sp.	4-7 × 8-21	2	0
<u>7</u>	Saccharomyces sp.	1-6 × 1-20	<u>10</u>	<u>0</u>
11			15	0
<i>Small spore sizes</i>				
3	Oospora sp.	2-5 × 8-18	4	0
<u>2</u>	Hemispora sp.	2 × 5	<u>2</u>	<u>0</u>
5			6	0
Subtotals				
43			62	0
<i>Unknown spore sizes</i>				
3	Other fungi (unidentified)		5	0
Totals				
46			67	

*Sequential impaction cascade sieve volumetric air sampler.

DISCUSSION

Comparison of the GSC plate and the SICSVA or Andersen sampler is valid only from a few limited perspectives because of the considerable differences between the 2 methods.

Some areas of incomparable contrast include the initial and operating costs, amount of medium required per sample, accuracy of controlling the sampling time, type of air flow contact with the sampling surface, determination of the volume of air sampled, cleanup and autoclaving requirements, size of required incubation area, operational complexity, and the ability to classify the sampled aerosols by particle size.

Both methods, when used for microbiological sampling, are adversely affected by extreme heat and dryness. Neither method is wind oriented and neither is suitable for isokinetic air sampling at various flow rates. The suggested flow rate for the Andersen sampler is one cubic foot per minute, while the random flow of air across the surface of a GSC plate cannot be accurately estimated. Neither method can be said to be designed for absolute aerosol recovery per unit of time.

Operational necessities common to both methods are medium and culture plate preparation and capability to grow, isolate, and identify the more common fungal genera.

The narrowly defined particle-size-collection ranges described by Andersen⁶ (see his Fig. 5) for each stage of his SICSVA sampler may be misleading. This arbitrary classification encourages the expectation that such ranges of particle classification by size are accurate. Flesch¹² has shown with monodispersed aerosols that there is overlap from stage to stage of the Andersen sampler with slippage of some large particles to smaller particle-collecting stages as well as collection of a few small particles in the stages which usually collect larger sizes. Our data (Chart IV; Tables II and III) show evidence of slippage and some skew of fungus spore distribution by size; but generally the colonies deriving from large spores are found in the non-lung-penetrating Stages 1 and 2, and the genera originating from small spores, small enough to be lung penetrating, do collect mainly in Stages 3, 4, 5, and 6, with intermediate sizes not localized.

Less than precise aerodynamic classification of particles by sizes may not be the only reason for variation in the distribution of fungal colonies in the various stages of the SICSVA sampler. Aggregates or clumping of fungal spores might be a partial reason why quite a few colonies of small spore-sized fungal types such as *Hormodendrum* and *Penicillium* were found on Stages 1 and 2. Single or clustered fungal spores riding as passengers on larger masses of particulate matter could be another explanation.

Knowledge of the spore size of microbiological aerosols additionally permits estimation of the gravity-settling rate of the collected particles. It is reasonably accurate to predict that the spores identified in Stages 3, 4, 5, and 6 of the SICSVA sampler will remain buoyant and thus airborne longer than the larger-sized spores so commonly found in Stages 1 and 2. The significance of this classification of collected airborne fungi by spore size, unique to the Andersen sampler, must be determined by future studies.

Greater numbers of large-, intermediate-, and small-sized spores resulting in colonies of more different fungal types were collected more frequently by the SICSVA sampler than by the GSC plate (Charts I, II, and III; Table I).

Though the 7 different genera or fungal types found only by the SICSVA sampler were a small percentage of the total colonies, they could, individually, be quite significant to the allergist trying to correlate a positive skin test or select for treatment or direct challenge the fungi in the air the patient breathes.

A striking example of the greater efficiency of the Andersen sampler was the percentage of positive isolations by fungal category (Chart II). *Rhizopus* was found on 29 occasions by the SICSVA sampler but only once by the accompanying GSC plates. *Alternaria* was isolated on 53 of the 127 tests (42 per cent) by the SICSVA sampler but on only 19 occasions (15 per cent) by the GSC plate. *Penicillium* was located by the SICSVA sampler in each of the 127 tests but by only 50 of the accompanying GSC plates (39 per cent). The importance of this difference may be highlighted by restatement: *Penicillium* was always present in the air when the Andersen sampler was used but absent

61 per cent of the times the GSC plate was employed. The misleading capability of the GSC plate as compared to the SICSVA sampler is further emphasized: *Hormodendrum* was absent from the air in only 2 per cent of SICSVA samples but in 24 per cent of the GSC plates; *Aspergillus* was absent in 7 per cent of the SICSVA samples but in 76 per cent of the GSC plates, etc.

On 8 of the 127 sampling occasions, the GSC plate wrongly indicated that the air was totally free of fungal growth, but 296 colonies of 12 different genera were identified from the same air by the accompanying SICSVA samplers. All instances of falsely negative isolations by the GSC plate (Chart V) were approximately 12 times more frequent than the reverse (falsely negative SICSVA sampler but positive GSC plate, Chart VI). The differences in colonies of types falsely reported absent from the air by the GSC plate were even greater: a little more than 60 times more colonies found by the SICSVA sampler with negative GSC plate for certain genera than the converse.

Thus, when used to indicate the presence or absence of a particular fungal type, the error demonstrated in this study by the GSC plate is enormous when this method is compared to the Andersen sampler. Different results might be obtained under different conditions of comparison such as extending the time of exposure of the open GSC plate⁵ or blowing air onto the surface of an open GSC plate.

These great differences, though from limited perspectives, clearly illustrate the advantages of the SICSVA or Andersen sampler and are probably due to its capacity to estimate volumetric concentrations of viable particles in a reproducible fashion from time to time and from place to place.

The user of the simple, inexpensive GSC plate to estimate quantities of airborne fungal flora must consider the multitude of falsely negative data associated with this very inaccurate method as it is usually employed. Such inaccuracies should be of special importance to the allergist attempting to assay a particular climate, assess his efforts at producing inhalant allergen-free air, or select, for direct challenge or treatment, the fungi present in the air of the patient's environment.

This study amply supports the contention⁴ that the GSC plate should not be used for the quantitative estimation of airborne fungal flora. The superior efficiency of the Andersen or SICSVA sampler for this purpose is demonstrated.

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REFERENCES

1. Morrow, M. D., Meyer, G. H., and Prince, H. E.: A summary of airborne mold surveys, *Ann. Allergy* **22**: 575, 1964.
2. Goodman, D. H., Northey, W. T., Leathers, C. R., and Savage, T. H.: A study of airborne fungi in the Phoenix, Arizona, metropolitan area, *J. Allergy* **38**: 56, 1966.
3. Goldfarb, A. A.: The significance of mold spore allergy in childhood respiratory disease, *Ann. Allergy* **26**: 321, 1968.
4. Wolf, H. W., Skaliy, P., Hall, L. B., Harris, M. N., Decker, H. M., Buchanan, L. N., and

- Dahlgren, C. M.: Sampling microbiological aerosols, United States Department of Health, Education, and Welfare, Public Health Service Monograph 60, 1964, pp. 9, and 39.
5. Cole, W. R., and Bernard, H. R.: Quantitative air sampling. A contrast with the settling-plate method for the study of bacterial air contamination in operating rooms, *Surgery* 51: 658, 1962.
 6. Andersen, A. A.: New sampler for the collection, sizing, and enumeration of viable airborne particles, *J. Bact.* 76: 471, 1958.
 7. Sheldon, J. M., Lovell, R. G., and Mathews, K. P.: A manual of clinical allergy, Philadelphia, 1967, W. B. Saunders Company, pp. 338-339.
 8. Hall, L. B.: Air sampling for hospitals, *Hospital Topics*, 40: 97, 1962.
 9. Gregory, P. H., and Lacey, M. E.: Isolation of thermophilic actinomycetes, *Nature* 195: 95, 1962.
 10. Hoggan, M. D., et al.: Isolation of *Coccidioides immitis* from the air, *Stanford M. Bull.* 14: 90, 1956.
 11. Davies, C. N.: Inhaled particles and vapours, Oxford, 1961, Pergamon Press, Inc., pp. 309, 466.
 12. Flesch, J. E., Norris, C. H., and Nugent, A. E.: Calibrating particulate air samplers with monodisperse aerosols: application to the Andersen cascade impactor, *Am. Indust. Hyg. A. J.* 28: 507, 1967.