

Improved techniques for sampling airborne fungal particles in highly contaminated environments

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BLOMQUIST G, PALMGREN U, STRÖM G. Improved techniques for sampling airborne fungi particles in highly contaminated environments. *Scand J Work Environ Health* 10 (1984) 253–258. Nuclepore filters (polycarbonate) were compared with a slit sampler for the collection of airborne fungi particles. To enable high spore-concentration sampling, the slit sampler method was modified by the use of collection gels consisting of agar or glycerol/gelatin. The exposed gels were homogenized (agar) or melted (glycerol/gelatin), diluted, and spread on cultivation media. No significant difference was found between the Nuclepore filter method and the slit sampler method when glycerol/gelatin gels were used in the interval 10^3 – 10^8 colony-forming units (cfu) per cubic meter. The lower trapping efficiency with agar gel was probably caused by water evaporating from the medium. The efficiency of a personal cascade impactor (Sierra) was compared with that of the Nuclepore filter method. The collection media used in the personal cascade impactor was modified for the determination of colony-forming units by moistening the collection filters with glycerol. The exposed filters were extracted with sterile water, and the suspension was diluted and spread on cultivation media. In trapping efficiency, the personal cascade impactor was similar to the Nuclepore filter method. The cut point of the personal cascade impactor showed high conformity with that of an Andersen sampler.

Key terms: high concentrations, Nuclepore filter, personal cascade impactor, slit sampler, spore.

Much interest has been focused during the past decade on problems caused by microorganisms in the work environment (2, 18). It is well known that high concentrations of bacteria and fungi can cause an allergic lung disease called extrinsic allergic alveolitis (8, 11). This phenomenon calls for methods to register the levels of microorganisms present in the air. It is also of interest to determine whether methods used to diminish the presence of microorganisms in the work environment are successful.

Commercially available sampling devices use different techniques to trap airborne microorganisms. Collection can be performed on agar gels (cascade impactors and slit samplers), in liquids (impingers and cyclone samplers), and on filters (6, 7, 14). Most of these sampling devices are designed for use in indoor environments, such as hospitals and offices (3, 17). In such cases the levels rarely exceed 10^4 microorganisms/m³ of air, while work with moldy grain or wooden fuel chips involves concentrations often exceeding 10^7 spores/m³ of air (10). Such instruments as cascade impactors or slit samplers, which collect the microorganisms on an agar gel, cannot be

used without modification in environments with high concentrations of microorganisms because the agar plates would become overloaded with fungal spores and bacteria and the estimation of colony-forming units (cfu) would be impossible. To solve this problem, the collection gel used in the slit sampler can be homogenized, serially diluted, and spread on an agar gel for cultivation (4). The cyclone developed by Errington & Powel (9) is an example of a device which traps microorganisms in a liquid. The liquid can be serially diluted prior to analysis.

The trapping efficiency of membrane filters has been studied in a number of investigations (5, 16). The microorganisms collected on the filters can be washed and diluted prior to analysis. The use of newer types of filters (Nuclepore) which collect the microorganisms exclusively on the filter surface should enable more accurate determination.

The purpose of the present investigation was to develop suitable methods for enumerating airborne microorganisms in environments with very high concentrations and to evaluate methods for determining mold fungi spores in the breathing zone of workers.

Materials and methods

Sampling devices

The collection of fungal spores was performed with a slit sampler (Casella Bacterial Sampler Mk 2, CF Casella & Co Ltd, London, England), a Nuclepore filter [Nuclepore Corp, United States (USA)], and the following two cascade impactors: (i) a 290 Marple

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Personal Cascade Impactor (Sierra Instruments, Inc, Carmel Valley, California, USA) and (ii) an Andersen sampler (Andersen Samplers and Consulting Service, Provo, Utah, USA) (1).

Sampling methods

Slit sampler method. Two types of media were used for the collection of fungal spores, one consisting of 1.5 % [weight/weight (w/w)] purified agar (Oxoid L 28) and the other of 7.1 % (w/w) gelatin (Merck) and 50 % (w/w) glycerol. Petri dishes (diameter 90 mm) were filled with 8-ml portions of the two collection media. The amount of air drawn through the sampler was 30 l/min. After the sampling period, the exposed agar gels were removed from the petri dishes and homogenized as previously described (4). The exposed gelatin glycerol media were melted in an adjustable thermostat cultivator at 40°C for 5 min and diluted with a sterile aqueous solution of 0.1 ‰ (w/w) Tween 80. The homogenized and dissolved gels were serially diluted and spread on cultivation media.

Nuclepore filter method. Nuclepore filters with a pore size of 0.4 μm and a diameter of 37 mm were placed on cellulose support pads and sealed in presterilized plastic filter cassettes (Millipore Corp, USA). The filter holders were connected by rubber tubing to portable constant-flow pumps (Universal Constant-Flow Sample Pump, SKC Inc, USA) calibrated for an airflow of 1 l/min. After the sampling period, the inlet and outlet connections of the filter cassettes were plugged and brought to the laboratory for analysis. The extraction of mold fungal spores was performed as follows: One milliliter of sterile water containing 0.1 ‰ (w/w) of Tween 80 was added to the cellulose support pad through the outlet connection of the cassette. After the connection was plugged, 5 ml of the liquid was pipetted through the inlet connection, whereupon the filter cassette was plugged and shaken for 15 min on a shaking table. After the extraction procedure, the fluid was sucked off with a pasteur pipette, serially diluted, and plated out on cultivation media.

Cascade impactor methods. A six-stage Sierra Marple Personal Cascade Impactor, with particle size cut points of 10, 6, 3.5, 2, 1, and 0.6 μm at 2 l/min, was loaded with Mylar medium collection substrates (diameter 34 mm). To produce an adhesive surface on the collection substrates, the Mylar media were moistened with sterile glycerol. The cascade impactor was connected by rubber tubing to a portable constant-flow pump, calibrated for an airflow of 2 l/min. At the end of the exposure period, the collection substrates were removed from the cascade impactor and separately extracted with 5 ml of sterile water containing 0.1 ‰ (w/w) of Tween-80. The

extraction fluids were serially diluted and spread on cultivation media.

The six-stage Andersen sampler with cut points of 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65 μm was loaded with petri dishes, each filled with a 27-ml portion of cultivation medium. During the air sampling period, 28.3 l/min was drawn through the sampler by a pump.

Cultivation conditions

After preparation, the samples collected with a slit sampler, Nuclepore filter, and the Sierra personal cascade impactor were plated out on a cultivation medium consisting of 1.5 % (w/w) purified agar (Oxoid L 28), 2.5 % (w/w) malt extract (Oxoid L 39), and 30-mg/l streptomycin sulfate. After 4 d of incubation at 20°C, the number of viable colonies was determined. The agar plates from the Andersen cascade impactor were incubated without further preparation.

Experimental procedures

Airborne fungal particles were collected in farm buildings, where work with moldy grain was in progress, in storehouses and heating plants, where wooden fuel chips were being handled, and in a potato warehouse. A comparative study of the different sampling devices was performed according to the following procedures:

1. In an examination of the surface properties of the collection gels used in the slit sampler, 8- and 20-ml portions of the two collection media were exposed for a 60-min period. The loss in weight due to water evaporating from the gels during the experimental period was ascertained.
2. Nuclepore filters were run parallel with the slit sampler, both collection media being used. The time of exposure for the agar gels was 10–20 min, and for the glycerol/gelatin gels 30–60 min.
3. In a study of the sampling efficiency of the Sierra personal cascade impactor and the Nuclepore filter method, the two devices were run simultaneously for 19 series of 60 min each.
4. During 10 sampling periods with the Sierra personal cascade impactor, six 15-s runs were performed during each sampling period with the Andersen sampler for a comparison of the particle-size cut points of the two cascade impactors used.

Results

Figure 1 shows the comparative study of the evaporation of water from the traditional agar medium and the new type of collection medium consisting of glyc-

erol/gelatin and water. Petri dishes, each containing 8- and 20-ml portions of the two collection media were exposed in a slit sampler for a 60-min period. As the figure shows, the petri dishes not containing glycerol/gelatin rapidly lost weight over the sampling period because of evaporation. At the end of exposure, the 20-g and 8-g glycerol/gelatin gels had lost about 10 and 20 %, respectively, in weight. These values correspond to a water loss of 20 and 40 %, respectively, since the water content in these gels is only 50 % of that in the agar gel. In the same experiment the agar gel lost 45 % of its water content when 20-g gels were used, and 90 % when 8-g gels were used.

In figures 2 a and 2 b the trapping efficiency of the two types of slit sampler collection media is compared with the efficiency of the Nuclepore filter method. The correlation coefficient (0.99 for glycerol/gelatin gel and 0.97 for agar gel) and the slope of the regression lines show a high degree of conformity between the methods in the range 10^3 – 10^8 cfu/m³ of air. A slight decrease in the trapping efficiency of the agar gel in comparison with that of the glycerol/gelatin gel was noticed, however, when very high concentrations of airborne spores of mold fungi were collected.

Figure 3 illustrates the trapping efficiency of the Nuclepore filter method as compared with the modified Sierra personal cascade impactor method. The concentration of airborne fungal spores varied between 10^3 and 10^8 cfu/m³ of air. As shown in the figure, there is good correlation (0.97) between the number of spores registered by the two methods.

Table 1 shows a comparison between the personal cascade impactor and the Andersen sampler at a similar cut point (3.4 μ m). The sum of viable counts in stages 3–5 was compared with the total amount of spores collected. The differences in size distribution were small, despite the fact that the results obtained from the Andersen sampler was an average of six 15-s

runs performed during 1 h, compared with a sampling time of 1 h for the Sierra personal cascade impactor.

Discussion

The use of a slit sampler as a sampling device for the collection of airborne microorganisms may be inaccurate when the evaporation of water from the collection medium occurs. This problem grows when longer sampling periods are used for the accurate determination of airborne microorganisms. The evaporation of water from the collection medium increases the distance between the agar surface and the slit. This increase, in turn, affects the trapping efficiency of the sampling device. The use of oxyethylenedocosanol (OED) has been suggested to prevent this desiccation (13). Trapping efficiency is also affected by a decrease in the stickiness of the trapping medium.

The results in figure 1 show that a modified collection media of glycerol/gelatin and water diminished the evaporation of water. When the surface properties of the exposed gels were examined, it was found that the stickiness of the glycerol/gelatin gels did not decrease during the experiment and it was even higher at the end of exposure than at the beginning.

In previous studies the use of carboxymethyl cellulose as an additive to agar gels has been suggested for cold-temperature air sampling (12). This additive is not necessary however when glycerol/gelatin gels are used.

The conventional method of using a slit sampler, that is, by incubating the exposed collection gels without further preparation, creates difficulties when air samples are collected in environments with high concentrations of mold spores. These problems arise because even short sampling periods (30 s) result in overloaded agar plates. The colonies developing on

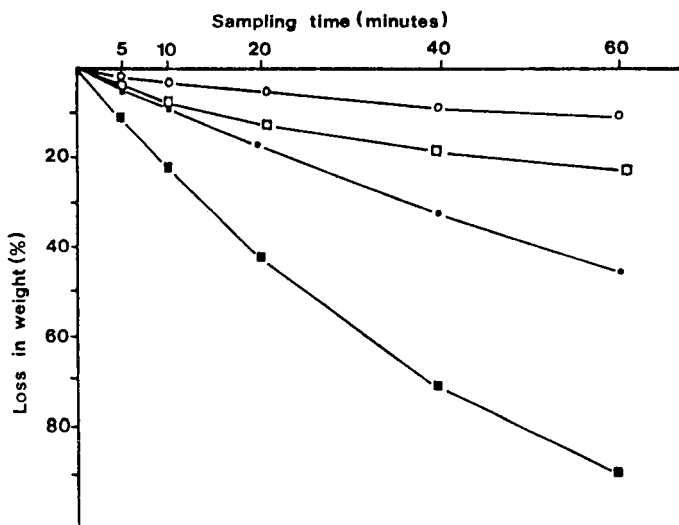


Figure 1. Loss in weight due to the evaporation of water from the collection gel during air sampling with a slit sampler. (○—○ = 20 g of glycerol/gelatin gel, □—□ = 8 g of glycerol/gelatin gel, ●—● = 20 g of agar gel, ■—■ = 8 g of agar gel)

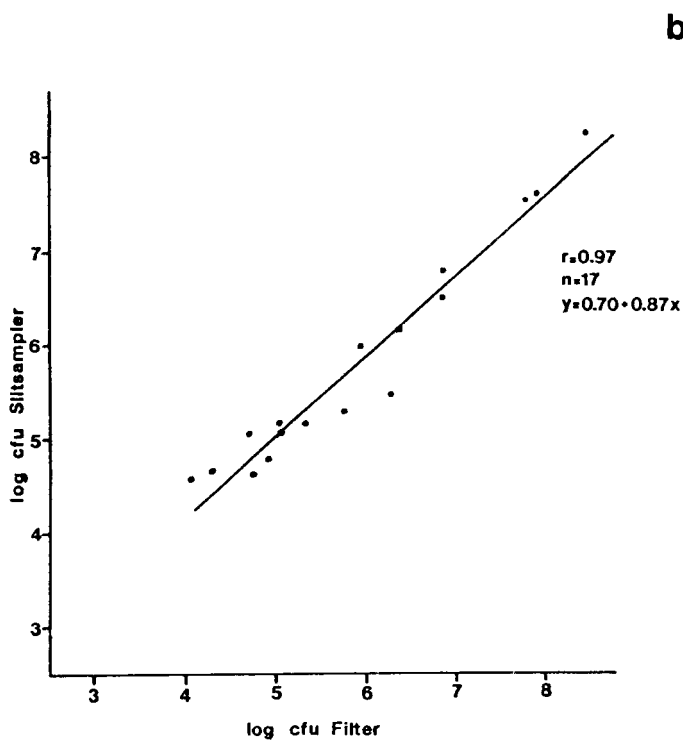
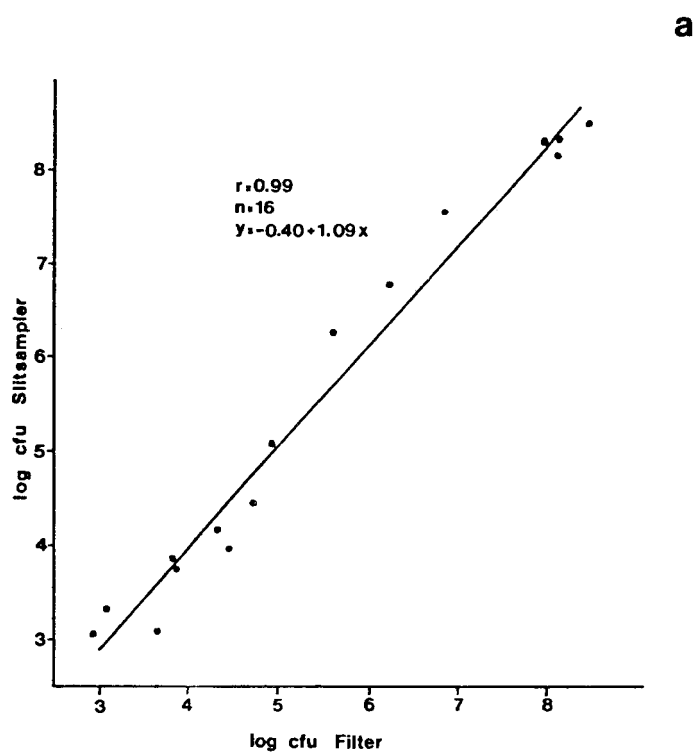


Figure 2. Comparison of the sampling efficiency of the Nuclepore filter method and the modified slit sampler method using glycerol/gelatin gel (a) and agar gel (b). (cfu = colony-forming units)

an overexposed agar gel cover the entire surface area and are thus impossible to estimate. In addition, metabolites may be produced which inhibit the development of neighboring colonies. This occurrence

prevents a realistic estimation of the number of fungal propagula. A previous investigation (4) showed that a small modification of the slit sampler method, including homogenization of the exposed

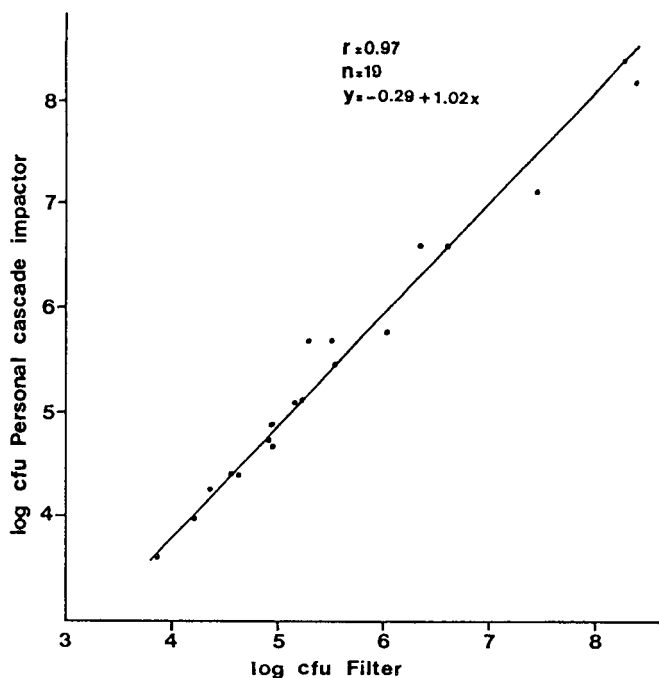


Figure 3. A comparison of the sampling efficiency of the Nuclepore filter method and the modified Sierra personal cascade impactor method. (cfu = colony-forming units)

agar gel to enable dilution of the sample, makes it possible to register very high concentrations of spores of mold fungi. To avoid the homogenization procedure, a new collection medium consisting of glycerol/gelatin and water was developed. In this paper, the modified slit sampler method, using the two types of collection media, is compared with the Nuclepore filter method (15).

The Nuclepore filter and slit sampler methods showed similar trapping efficiency in the range 10^3 – 10^8 cfu/m³ of air, although the use of agar gel as a collection medium tended to give lower counts above 10^7 cfu/m³ of air. As was already stated, the lower trapping efficiency in this case was probably due to the evaporation of water from the agar gel. The results further indicate that the high concentration of glycerol (50 % in the collection gel) did not influence the viability of the trapped fungal spores. Since the collection gel is melted and suspended in sterile water, the relative concentration of glycerol is markedly reduced before it is spread on the cultivation plates.

In view of the weight and dimensions of the apparatus, the slit sampler method is limited to stationary emission studies, while the Nuclepore filter method is suitable — apart from stationary sampling — as a personal sampler for collection in the breathing zone.

Stationary air sampling for detecting microbial emission sources does not always reflect hazards in the work environment. Personal sampling devices for collection in the breathing zone are important for a more accurate risk appraisal. For this purpose a new, commercially available personal cascade impactor

Table 1. Comparative study of the 3.5- μ m cut point of two types of cascade impactors. The results were obtained from a comparison of the sum of viable counts on stages 3–6 of the cascade impactors with the total number collected.

Test number	Percentage of colony-forming units on stages 3–6 compared with the total number of colonies	
	Andersen sampler	Sierra personal cascade impactor
1	89	58
2	62	46
3	69	69
4	70	68
5	49	49
6	69	86
7	81	78
8	86	89
9	81	78
10	84	93

was compared with the Nuclepore filter method. The collection filters of the cascade impactor was modified as stated in the Materials and Methods section to enable an estimation of viable counts. The results showed that the trapping efficiency of the filters moistened with glycerol was equal to the Nuclepore filter method when both high and low concentrations of airborne fungal particles were sampled. Nor in this investigation did the high concentration of glycerol affect the viability of the trapped conidia. Since glycerol is soluble in water to a certain extent, no problems were encountered in the washing of the collected conidia from the pretreated cascade impactor filters.

In an examination of the size-fraction characteristics of the personal cascade impactor, parallel runs

were performed with an Andersen cascade impactor. To avoid overexposed agar plates, parallel runs could only be performed in the range 10^3 – 10^5 cfu/m³ of air. When the two types of equipment were compared with the use of a similar cut point ($< 3.5 \mu\text{m}$), there was high conformity between the two. The significantly lower yields in some of the tests may have been due to the inhomogeneity of the concentrations of fungal particles in the air during the sampling period.

The disadvantage of using the personal cascade impactor, as compared to the use of the Nuclepore filter method, is the time-consuming preparation of the equipment prior to sampling. The advantage of cascade impactors, compared with other sampling devices, is that they can fractionate the airborne microorganisms and thus provide information on size distribution. This information may also be obtained by the Nuclepore filter method if acridine orange staining is combined with size analysis of the collected particles in an epifluorescence microscope (19).

All of the sampling devices studied in this investigation are useful in highly contaminated environments. For personal sampling and stationary emission studies the Nuclepore filter method was found to be the most practical.

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