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Sampling of high concentrations of airborne fungi

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BLOMQUIST G, STRÖM G, STRÖMQUIST L-H. Sampling of high concentrations of airborne fungi. *Scand J Work Environ Health* 10 (1984) 109–113. The paper discusses a method developed for determining airborne fungi particles in environments highly contaminated with mold fungi. The collection of airborne fungi was performed with two slit samplers. These sampling devices were found to give the highest values out of the three different types tested simultaneously. After spores had been collected with the slit samplers, the collection medium, an agar gel, was removed from the petri dishes and homogenized in a sterile 0.9 % sodium chloride solution. The homogenate was diluted stepwise and spread on agar plates prior to the cultivation and the determination of viable counts. When the homogenization procedure was tested on samples collected from three different work environments, no increase or decrease in the number of colony-forming units could be detected. Storage of the homogenate at 2°C over 9 d did not increase the number of viable fungal colonies.

Key terms: cascade impactor, cyclone, slit sampler, spores.

In recent years awareness has grown of problems caused by pollution due to fungal conidia and actinomycetous spores in the work environment. In sawmills allergic lung diseases are known to be caused by airborne spores (1, 7). A similar problem arises in the handling of moldy hay by farmers (2, 5). Health problems caused by airborne conidia and spores have been noted in several other work environments (4, 6).

Since high concentrations of conidia and spores in the air can cause health problems, it is necessary to have reliable methods for recording the levels of occurrence in the air. Reliable methods are important for assessing whether a health hazard exists and also for determining whether any remedial actions taken have resulted in lower concentrations.

In the determination of the amount of airborne conidia or spores, the choice of method has a great bearing on the result. It is necessary to sample in a reproducible and representative manner, without changing the characteristics of the microorganisms. If the fungi are to be studied after collection, it is often essential that they be alive. The sampling of spores and conidia can be carried out with different methods, of which the most common is impaction on gels, tapes, or liquids. It is even possible to use filtration in many cases. The sampling procedures are very similar to those used in ordinary dust sampling. However, conidia and spores, being living particles, are more fragile.

Most of the sampling devices described in the literature have been used in environments with low concentrations of airborne microorganisms. When the concentration exceeds about 10^5 spores/m³, difficulties arise in evaluating the sample. When a cascade impactor or slit sampler is used at such concentrations, too many spores or conidia are collected on the gel, even if short sampling times are selected. The aim of the present study was to develop a method for accurately determining high concentrations of airborne conidia through a simple improvement in the slit sampler technique. The modified method should enable sampling concentrations above 10^6 conidia/m³ in environments where allergic alveolitis may be suspected to occur. The method was evaluated through comparison with the traditional slit sampler technique, as well as comparison with other available methods, such as those using a cascade impactor and a cyclone sampler. The possibility of storing the collected air samples at a low temperature was also investigated.

Materials and methods

Sampling devices

Fungal conidia were collected with slit samplers (Casella bacterial sampler Mk 2, CF Casella & Co Ltd, London, England; BIAP slit sampler, Mikrobiologiska och Biotekniska Testprodukter AB (MBT AB), Malmö, Sweden), a cascade impactor (Andersen sampler, Andersen Samplers and Consulting Service, Provo, Utah, the United States), gelatin filters (Sartorius membrane filter 3.0 µm), and a cyclone sampler. The experiments with the cyclone sampler were kindly performed by E Henningsson of the National Defence Research Institute, Umeå, Sweden. The cyclone used was developed by Errington & Powel (3).

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Slit sampler (traditional method)

Petri dishes with a diameter of 14 cm, loaded with cultivation media, were placed in a slit sampler. The medium was composed of 1.5 % (weight per weight) purified agar (OXOID L28), 2 % (weight per weight) malt extract (OXOID L39), and a 30-mg/l concentration of streptomycin sulfate (Sigma Chemical Co, St Louis, Missouri, the United States). The distance between the agar surface and the slit was adjusted. The air flow through the sampler was 30 l/min, and

the sampling period was 10 min. After exposure the petri dishes were incubated at room temperature (20–22°C) for 4 d.

Slit sampler (modified method)

The collection medium used in the slit sampler consisted of 1.5 % (weight per weight) purified agar (OXOID L28); 8- and 16-ml portions of the medium were poured into petri dishes, with a diameter of 9 and 14 cm, respectively. After exposure the collection media were removed from the petri dishes and homogenized with an Ultraturrax T18/10 homogenizer (Janke & Kunkel GmbH & Co, Staufen, Federal Republic of Germany) for 20 s in a 0.9 % sterile sodium chloride solution to a total of 40 g when the 8-ml gels were used and 80 g when the 16-ml gels were used. After preparation the samples were diluted stepwise and spread on the cultivation media. Part of the undiluted homogenate was stored at 2°C and, after the determination of the colony-forming units (cfu), plated out in a narrower interval. After 4 d of cultivation at room temperature (20–22°C), the number of colony-forming units were determined.

Cascade impactor method

A six-stage Andersen sampler was loaded with petri dishes containing cultivation medium. To achieve the right distance between the agar surface and the sieve plates, 27 ml of cultivation media was poured into each petri dish. The air flow through the sampler was adjusted to 28.3 l/min, and the sampling period was 15 s. After the exposure the cultivation plates were incubated as already described.

Cyclone sampler method

The cyclone sampler was operated according to Errington & Powel (3). The sampling fluid used in the experiments consisted of 0.364 g of tris-(hydroxymethyl)aminomethane, 50 ml of glycerol (80 %), one droplet of Polyglycol P-2000 (Dow Chemical), and distilled water to 1,000 ml. The pH was adjusted to 7.2 with either 2 M sodium hydroxide or 1 M hydrogen chloride. The air flow through the sampler was 75 l/min, and the sampling period 10 min. At the laboratory the samples were diluted stepwise, spread on the cultivation media, and incubated as already described.

Experiment

Air samples were taken at a potato storehouse over a three-month period. The sampling devices were placed close to each other, at about 70 cm above floor level. The relative positions of the sampling devices were changed when repeat samples were taken. The following experiments were performed:

1. For a comparison of the traditional way of using the slit sampler (incubating the exposed agar gel without further preparation) with the modified

Figure 1. Comparison of traditional and modified slit sampler methods for determining the number of airborne fungal spores. (cfu = colony-forming units, BIAP = BIAP slit sampler)

Figure 2. Comparison of the sampling efficiency of two slit samplers using the modified method. (cfu = colony-forming units, BIAP = BIAP slit sampler)

Table 1. Number of airborne spores collected on gelatin filters in three different work environments and registered before and after homogenization with an Ultraturrax homogenizer. In each environment the spores were collected at two different concentrations.

Work environment	Colony-forming units/m ³ before homogenization		Colony-forming units/m ³ after homogenization		Number of experiments
	Mean	Standard error	Mean	Standard error	
Potato storehouse	5.9×10^4	0.2×10^4	5.8×10^4	0.2×10^4	10
	1.3×10^5	0.1×10^5	1.2×10^5	0.1×10^5	10
Wood trimming department	6.3×10^4	0.3×10^4	6.5×10^4	0.4×10^4	10
	1.2×10^5	0.1×10^5	1.3×10^5	0.1×10^5	10
Fuel-chip storehouse	6.3×10^4	0.3×10^4	6.5×10^4	0.4×10^4	10
	1.8×10^8	0.1×10^8	1.6×10^8	0.1×10^8	25

method, parallel runs were performed with two BIAP slit samplers.

2. In an investigation of whether the homogenizing procedure had any effect on the number of colony-forming units registered, air samples from three different work environments (a potato storehouse, a wood trimming department, and a wood fuel-chip plant) were collected on gelatin filters. Air (150 l) was drawn through the filters. The exposed gelatin filters were dissolved in 40 g of 0.9 % sodium chloride solution. Half the suspended filter solution was homogenized for 20 s. Both the treated and untreated suspensions were then plated out on the cultivation media.

3. To study the effects of low-temperature storage on the homogenate, the following experiments were performed. Airborne fungal spores collected on an agar gel were homogenized as described and stored at 2°C in a refrigerator for 10 d. During storage, small samples were taken every 24 h. The samples were diluted and plated out on the cultivation media as described.

4. For an evaluation of the modified slit sampler method, parallel runs were performed with two slit samplers, the cyclone sampler, and the cascade impactor. The samples were brought to the laboratory for analysis within 3 h of exposure.

Results

The comparative study of the two BIAP slit samplers using the traditional and modified methods is presented in figure 1. As this figure illustrates, there was a poor correlation between the two techniques (correlation coefficient 0.56), and the number of spores registered by the traditional method was less than the number registered by the modified method.

The results of parallel runs with the BIAP and Casella slit sampler and the modified method are shown in figure 2. Unlike the results described of the preceding comparative study, the correlation between the two slit samplers was good (correlation coefficient

Figure 3. Low-temperature storage (2°C) of the homogenized slit sampler collection media. The spores were collected with a slit sampler (Casella) at a potato storehouse. The vertical bars express the standard error. Each point represents an average of 36 cultivation plates. (cfu = colony-forming units)

0.99), and no significant difference in sampling efficiency was noted between the two samplers.

The modified method includes homogenization and dilution of the exposed agar gel. If aggregates of conidia are present in the sample, homogenization could eventually lead to an overestimation of the colony-forming units/m³. Therefore samples from three different work environments (a potato storehouse, a wood trimming department and a wood fuel-chip plant) were collected at two different concentrations of airborne conidia. The samples were analyzed as described in the Material and Methods section. The effects of the homogenization procedure are presented in table 1. No statistically significant increase or decrease in colony-forming units could be detected in the samples collected after the homogenization procedure.

Table 2. Comparison of the efficiency of the methods used for sampling fungal conidia. The analysis of the collection media used in the slit samplers was carried out according to the modified method described in the Material and Methods section. The Casella slit sampler was given 100 %. (N = number of comparisons)

Sampling device	Spores/m ³							
	10 ³ —10 ⁴		10 ⁴ —10 ⁵		10 ⁵ —10 ⁶		10 ⁶ —10 ⁷	
	%	N	%	N	%	N	%	N
Slit sampler								
Casella	100	13	100	41	100	20	100	14
BIAP	87	13	100	41	71	20	92	14
Cyclone	81	13	80	41	45	20	33	7
Cascade impactor	72	10	73	10	.	.	.	.

Figure 3 shows the results of the cold-temperature storage of the homogenized suspension. No significant increase in the number of colony-forming units seemed to occur during the first 9 d of the experimental period. However on the tenth day there appeared to be an increase. Therefore the samples were never stored more than 4 d.

Table 2 shows the comparison of the collection efficiency of the two slit samplers, a cascade impactor, and a cyclone sampler at four different spore concentrations. The collection media of the slit samplers were analyzed according to the modified method.

The sampling efficiency of the Casella slit sampler was set at 100 % in comparison with the other methods. At the two lower levels, 10³—10⁴ and 10⁴—10⁵ spores/m³, a small but insignificant difference between the methods used was noted. At higher concentrations, 10⁵—10⁶ spores/m³, however, the cascade impactor could not be used because of overgrowth of the agar plates, despite the limitation of the sampling period to 15 s. As shown in the table, the cyclone sampler showed a decrease in sampling efficiency with increasing amounts of conidia in the air. The lower sampling capacity of the BIAP slit sampler at the range 10⁵—10⁶ spores/m³ may be explained by the limited amount of samples or inhomogeneities in the environment where the samples were collected. When all parallel samples were taken into consideration, no difference could be detected between the two slit samplers.

Discussion

In recent years much attention has been paid to extrinsic allergic alveolitis. This disease can be found to occur in environments which are highly contaminated with microorganisms. The traditional use of sampling equipment such as slit samplers and cascade impactors is impossible at these levels of airborne fungi particles since the agar plates would be covered with colonies even if the sampling time were markedly reduced. Our results show that, if a modified sample preparation procedure is followed, it is possible to use commercial sampling devices, such as slit samplers, to get

highly reproducible determinations. This modified sample preparation includes homogenization of the exposed collection gels for stepwise dilutions of the sample and low-temperature storage of the homogenate to enable preliminary scanning of the colony-forming units. The advantage of using the modified sample preparation procedure, as compared to the traditional slit sampler method, is its ability to collect airborne conidia in environments with very high concentrations without problems with overloaded agar plates. The poor correlation between the two sample analysis techniques described in figure 1 illustrates this problem. When the traditional method was used, even at low concentrations of airborne spores, the number of colonies developing on the exposed agar plates had to be determined at an early stage in development (2 d of cultivation), and a rough estimation of the number of colonies was made from a count on segments of the agar gel. The slower growing species could not be detected in such cases. Furthermore, when too many fungal colonies grow on an agar plate, the metabolites produced during growth may inhibit neighboring colonies. This phenomenon leads to an underestimation of the number of colony-forming units registered.

When the modified method was used, no such problems arose since the samples were diluted. When the two types of slit samplers were compared, the modified technique being used with both, a high correlation was achieved. It is a well known fact that mold fungi spores have a wide temperature range for growth. Some species can grow even at temperatures below 0°C, although the growth rates in these temperatures are highly restricted. The results from the low-temperature storage showed the possibility of keeping a suspension of homogenized agar in a refrigerator at 2°C for 9 d without an increase in the number of viable counts. This prolonged storage at a low temperature made preliminary dilutions and cultivations of the collected samples possible. After 4 d of incubation a brief determination of the number of colony-forming units was made, and new dilutions of the stored homogenate were carried out at an appropriate interval. In order to minimize the risk of

growth inhibition due to overloaded agar plates, when petri dishes with a diameter of 9 cm were used, only about 40 colonies were allowed to develop on a cultivation plate.

The homogenization of the exposed agar gels raises the question of whether the preparation procedure affects the viability of the conidia or whether an increase in the colony-forming units occurs due to the disruption of conidial aggregates or the fragmentation of active mycelia. The results of the present investigation showed that, when air samples collected on gelatin filters were subjected to homogenization, the number of colony-forming units was neither increased nor decreased in comparison with the corresponding number in untreated samples. This finding indicates either that no such aggregates or active mycelia exist to any great extent in the environments studied or that the homogenization procedure has no adverse effects on the collected fungal particles.

The former of the two possibilities is probably true, since the results in table 2 show no significant difference between the modified slit sampler method and the Andersen cascade impactor in the range 10^3 – 10^4 and 10^4 – 10^5 cfu/m³. The somewhat lower yields obtained with the cascade impactor, compared to those obtained with the modified slit sampler method, are probably due to the fact that the Andersen sampler results are an average of three 15-s runs during the 10-min exposure with the slit sampler. The time taken for the Andersen pump to work at full strength markedly affects the results when short sampling periods are used.

When the cyclone sampler was used, there appeared to be a decrease in the trapping efficiency at spore levels above 10^5 – 10^6 cfu/m³ of air in comparison to the level obtained with the modified slit sampler method. This phenomenon is probably caused by saturation of the sampling fluid with regard to fungi spores. Another explanation may be that the composition of the sampling fluid selectively extracts the spores according to their different hydrophobic surface characteristics.

Conclusions

The results show that it is possible to make an accurate determination of the number of fungal spores in the air, even at very high concentrations, with the modified slit sampler method. Neither the homogenization procedure nor the low-temperature storage influenced the number of colonies developing on the agar plate.

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