inoculum

Supplement to *Mycologia*
Vol. 55(5)
Sept. 2004

Newsletter of the Mycological Society of America

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Important Dates

October 15 Deadline:
Inoculum 55(6)

July 30-Aug. 5, 2005:
MSA-MSJ, Hilo, HI

August 15-19, 2005:
International Congress on the Systematics and Ecology of Myxomycetes V

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Limitations and Considerations in Air Sampling, Sample Analysis and Result Interpretation for Airborne Mould Spores

by Jackson Kung'u

Airborne mould material particularly spores are recognized allergens at normal concentrations to an estimated 20% of the population (Kendrick, 2000). It is not always possible to establish a correlation between mould spore counts in the environment and the presence of allergic symptoms. This has been partly attributed to the use of inadequate air sampling techniques and equipment (Sanchez & Bush, 2001). Mould spores elicit or exacerbate several types of respiratory conditions including allergic rhinitis and asthma. Sensitivity to fungi may develop depending on the individual resistance, amount of spores and the exposure time. Licorish, *et al.*, 1985, demonstrated that inhalation of either *Alternaria* or *Penicillium* spores in quantities comparable with those encountered by natural exposure could induce both immediate and late phase asthma in sensitive individuals. Thus, it is important to keep monitoring levels of airborne spore composition and concentration in indoor air. Monitoring of airborne concentration of pathogenic fungal spores has been used over the years in plant pathology to predict disease outbreaks and thus make rational decisions on whether to spray a crop or not. In indoor environment, air monitoring is not common except in hospitals and food and pharmaceutical processing plants. The need to determine airborne spore concentration and composition often arise when occupants complain of ill health.

Industrial and occupational hygienists may sample air for airborne fungal spores with the following objectives: (1) to determine the presence of airborne spores, their composition and concentrations in situations where occupants complain of ill health but with no obvious visible mould growth. This information could be used in assessing the possibility of hidden mould growth and human exposure; and (2) to determine if spores had become aerosolised from visible growth sources. The information obtained here could be used as background information in monitoring the effectiveness of remediation measures.

Specific objectives may include detection and quantification of certain mould species, for example, in hospital environments, the objective may include detecting the presence of *Aspergillus fumigatus, A. flavus* and *A. niger* which are causes of Aspergillosis in immuno-compromised patients.

Interpreting and drawing conclusions from results of airborne nonviable and viable fungal analysis can be difficult since there are no agreed occupational exposure limits (OELs) or threshold level values (TLVs) for mould. Despite lack of TLVs, spore traps are invaluable tools in indoor mould investigations. The objective of this article is to point out the limitations of air sampling, sample analysis and interpretations of spore trap results. This information, coupled with professional experience, would enable industrial hygienists and environmental consultants to interpret laboratory results and make rational decisions.

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Air Sampling Techniques

Sampling Devices. Efficient sampling of biological aerosols requires a clear understanding of the physical and biological attributes of the species under investigation. Biological attributes would also influence the choice of the culture medium in case of viable analysis.

There are various devices for air sampling for both viable and total spore counts (also referred to as non-viable) analysis (ACGIH, 1999). The collection efficiency of a device is dependent on flow rate and impaction principles, and the aerodynamic diameter of the particle. Aerodynamic diameter is the diameter of a sphere of density $1g/cm^3$ that has the same settling velocity as the actual particle under consideration (ACGIH, 1999). The aerodynamic diameters of fungal spores fall within the range 0.5µm to 20µm but typically larger than 2µm. Theoretically a sampling device will collect efficiently particles of sizes equal to or greater than the cut-off diameter.

Sampling Duration. Ideally the sampling period should be long, that is, several hours or days since the concentrations of airborne spores have spatial, temporal and diurnal variations. Therefore, short sampling durations only provide a snapshot of conditions. Unfortunately, for many devices, long sampling durations especially in contaminated environments, would result in overloaded samples that would be difficult to analyse.

Sample Volume. The sample volume is determined by the air flow rate and the sampling duration. The choice of sampling volume must be a compromise between obtaining a sample sufficiently large to be representative and other practical considerations such as avoiding overloading the sample. Often, prior knowledge on expected airborne particle concentrations is lacking and appropriate sampling durations and volumes must be selected through trial and error.

Sampling Height. Concentration of airborne spores varies with the height. Smaller spores are dominant at higher levels and larger spores are more prevalent at lower levels. In studying vertical profiles of outdoor spore concentrations, Chakraborty, *et al.*, 2001, found the spore counts for *Alternaria*, *Curvularia* and *Drechslera* highest at the height of 1 meter while those of *Aspergillus* spp, Basidiospores, and *Cladosporium* spp were highest at 5 meters. The variation in spore concentrations with height is influenced not only by the size/shape of spores but also by meteorological factors such as wind conditions, temperature, relative humidity, and precipitation.

Analytical Techniques

As mentioned earlier, spores could be trapped for viable analyses or for non-viable analyses (also referred to as total spore count). The two types of air samples differ in terms of the sampling media used, analytical methods and the results they give. Spores for viable analyses are usually trapped on growth medium (Figures 2a and 2b) while those for non-viable analyses are trapped on sticky surfaces or filter membranes. Both methods of spore trapping have some limitations. Selection of the type of samples to collect and the sampling media should be determined by careful consideration of the data required and how it would be interpreted. In some instances use of both non-viable and viable analysis would be recommended.

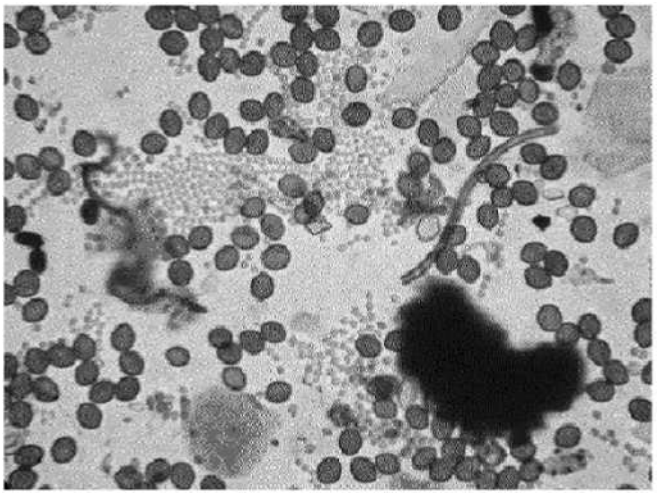

Figure 1a: Spores as seen under microscope. X630 (<0.06mm^2)

Total Spore Counts. Total spore count involves examining the spore deposition area under a microscope and counting deposited spores. The major advantage of this technique is that results can be obtained within the same day of sampling and counting does not depend on the viability of the spores. However, total spore counting has a number of limitations associated with the following:

Lack of Standardized Analytical Methods

To date there are no standardized methods for analysing spore traps. Some analytical laboratories count a portion of the spore trap as recommended by cassette manufacturers and then convert the counts to spores per cubic meter of air by simple proportions. However, this conversion may be highly inaccurate since spores are rarely uniformly distributed on the sample trace (i.e., the deposition of the spores on the slide is not random) and spores of some moulds tend to cluster together or form long entangled chains that may be difficult to count. Ideally the whole sample trace should be counted. Counting the whole spore deposition area is extremely difficult and time consuming if the slide is overloaded with fungal and non-fungal particulate matter. A field with a high particle density will easily result in analyst fatigue, with spores being overlooked or double counted.

The inherent biological characteristics of some mould spores such as formation of spores in clusters or chains not only present unpredictable non-random distribution of spores on the sampling media but also presents counting problems. Examples of moulds that form chains or clusters are *Penicillium*, *Aspergillus*, *Cladosporium*, *Paecilomyces*, and *Acremonium.* With no standardized counting procedures, opinions on how to count spores in chains or clusters vary (Groth, 1995; Groth, 1996a; Groth, 1996b; Muilenberg, et al, 1996). Some analysts count clusters or chains of spores as single units while others count each individual spore on the chain or cluster. Analysts who consider clusters or chains as single units reason that, these clusters or chains would form single colonies if the air were sampled for viable analysis. The other

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argument is that spores in clusters or chains would tend to inflate the counts if counted individually. However, a count based on the number of individual spores may be a better gauge of potential health risk. Figures 1a, 1b, and 1c illustrate the difficulties involved in counting spores in chains whether spores in chains are counted as individually or chains are counted as single elements.

Lack of consensus among analysts on spore counting technique makes it difficult for results from different laboratories and sometimes even individual analysts within the same laboratory to be compared. It is important that labs clearly outline their method of spore counting.

Nonhomogeneous Distribution of Spores in the Air

When converting raw spore counts to counts per cubic meter of air, the assumption made is that the spore distribution in the air is homogeneous which is very unlikely. Spore concentration is likely to be higher near the source than away from the source and may also vary with the sampling height (Chakraborty, *et al.*, 2001). This can lead to overestimation or underestimation of the actual spore concentration in the air and hence potential exposure to occupants.

Difficulties in Identifying Spores

Since some spores have no unique characters for identification, they are grouped together as unidentified spores. While sometimes the analysts are able to group the unidentified spores as basidiospores or ascospores, other times it is difficult to indicate what categories of spores are lumped together as unidentified. Reports from various commercial laboratories show great variability of the categories of moulds consistently reported by individual labs and not others which suggests that misidentification of mould spores is common.

Viable Analysis. Unlike non-viable analysis, viable analysis takes between 10-14 or more days for complete analysis. One major advantage of viable analysis is that it allows identification of mould to species level. This method is particularly appropriate in hospital environments where the objective of air sampling may be to estimate the concentrations of viable invasive or opportunistic pathogenic moulds. Another advantage of viable analysis is that if samples are not overloaded, colony counting and interpretation of results are generally easier. Limitations of viable analyses are due to:

Limited Spore Germination or Growth on Artificial Media. Most of the spores deposited on growth media will not germinate or continue to grow to recognizable colonies due to incompatibilities with the culture media or other growth conditions. It is estimated that less than 10% of all airborne spores would be culturable (Blomquist, 1994).

Some moulds such as species of *Trichoderma* may inhibit the growth of others. For these reasons, counts of colony forming units (CFU) are usually far much lower than total spore counts.

A single CFU on media could have developed from multiple fungal structures such as hyphal fragments and chains or clusters of spores. Fast growing moulds like *Aspergillus* and

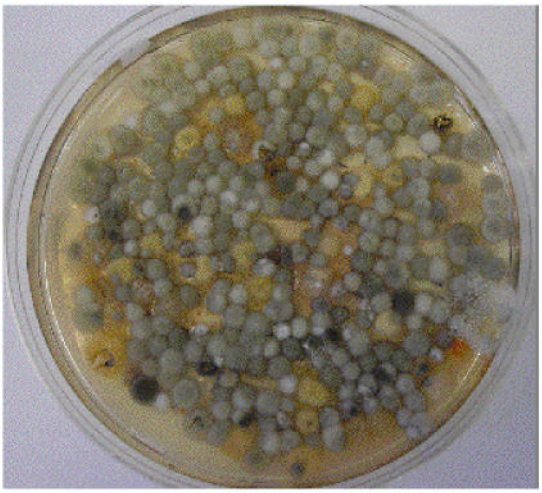

Figure 2a: Fungal colony forming units (CFUs) on agar medium in a petri-dish

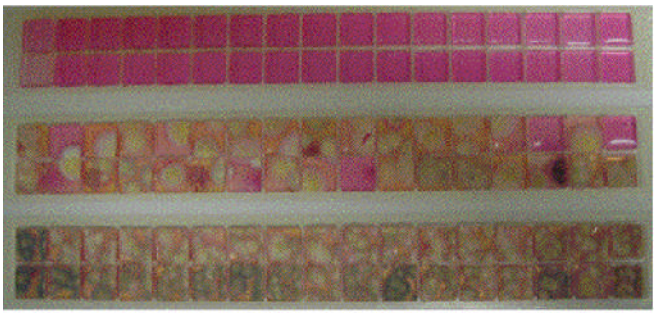

Figure 2b: Fungal colony forming units (CFUs) on agar medium in strips

Penicillium would also inhibit slow growing ones such as *Chaetomium* resulting to an underestimation of these moulds. Chang, *et al.*, (1994) demonstrated that colony counting efficiency decreased with increasing density of collected culturable micro-organisms, increasing colony size, and decreasing ability of an observation system to distinguish adjacent colonies as separate units.

In multiple hole samplers such as the Andersen and similar samplers a correction factor (also known as positive hole collection) is applied to give the probable statistical total CFU based on the number of holes in the sampling head and the colony counted. Tables have been prepared by the manufacturer which can give a value for the probable statistical total once the colonies have been counted (ACGIH, 1999). A correction factor is not applied on RCS samples.

Identification to Species. Identification of moulds is still largely based on morphological and cultural characteristics. Identification to species is extremely difficult and requires someone highly experienced in fungal taxonomy and nomenclature. Incorrect identification of moulds may result to expensive decisions being made. Currently, only a few commercial labs have the capability of identifying moulds to species particularly those belonging to the *Penicillium* and *Aspergillus* groups.

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Interpretation of spore trap results difficult due to two factors including:

Lack of Exposure Limits. Currently, there are no occupational exposure limits (OELs) for mould for comparing and interpreting laboratory results. Threshold level values (TLVs) for mould cannot be recommended for reasons which include limitations or variability in air sampling, variability of sensitivity to mould among the human population, occurrence of a large number of different types of biological and chemical indoor air pollutants, and limited data on exposure-response relationship. In absence of set TLVs indoor spore counts are compared with outdoor spore counts and composition. If the indoor levels of airborne mould are much higher than outdoor, or there are different species than outdoor, then it may be concluded that there is mould growing inside. If results indicate outside concentrations are much higher than inside, it may be concluded that there is no mould problem inside. However, there may be visible mould in the building while the air sampling indicated there was no significant problem. This can potentially happen if the mould had not been released spores due to the growth stage in its life cycle or something else could have happened to affect the sampling. A negative finding does not prove the absence of the hazard, but indicates only that the hazard was not detected. A combination of results of air samples and visual inspection is recommended in drawing conclusions. Comparison of indoor/outdoor samples may not be possible during winter or after heavy precipitation.

Lack of Clearly Defined Objectives. Lack of clearly defined sampling objectives for taking spore trap samples can make interpretation of results very difficult. The more specific the sampling objective is, the easier it is to interpret the results.

Conclusion

Despite the limitations, spore trapping (both for viable and total counts) is useful in air monitoring for biological agents. However, it is important that measures that would make the results meaningful and interpretable are taken. For example, before any sampling commences, there should be clear objectives for sampling and an understanding of how the results would be interpreted. Generally, air sampling may be conducted for qualitative or quantitative purposes. The aim of qualitative sampling is to determine the presence in rank order of some selected groups of moulds, while quantitative sampling aims at measuring the concentration of selected groups of moulds or no specific groups of mould.

Once the objectives of the sampling are well defined, it is then easy to decide whether to take samples for viable analysis, for total counts or for both. If the investigator is interested in determining the mould species present in the environment, the use of culturable-viable sampling is recommended since it allows for speciation. Viable analysis also provides an indication of how active in terms of spore production the source may be. Unlike total mould spore methods, concentrations on sample plates are more interpretable and easy to compare.

It is widely accepted that the total mould spore sampling is the most appropriate clearance sampling approach as all spores that are airborne have the potential for being collected and counted. The results are also obtainable within a shorter time (same day) if required. However, if the objective of the remediation was to reduce airborne viable spores such as in hospital environment, this may only be adequately demonstrated by use of viable spore traps.

The more specific the objective of sampling is, the easier it is to design a suitable sampling strategy and the easier it is to interpret the results. For example if the objective of sampling is to determine the presence and quantify airborne *Stachybotrys* spores, spore trapping for total counts would be the most efficient sampling strategy. However, a single sample may be inadequate to conclude that a building is free from airborne *Stachybotrys* spores.

Air sampling strategy must satisfy the objective of the investigation, be reasonably efficient at capturing the spores of interest and be compatible with required counting or analytical methods.

Lastly there is an urgent need for standardizing analytical methods for quantifying airborne mould spores so that results from different laboratories could easily be compared.

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