INVESTIGATING FUNGAL CONTAMINATION IN BUILDINGS

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Introduction

Exposure to fungal bioaerosols [such as spores, mycotoxins, volatile organic compounds (VOC's) and (1-3)-ß-glucan] in indoor air has emerged as a significant health concern in residential environments as well as in occupational settings, including offices and industrial sites (such as facilities for composting, wastewater treatment, sludge, and recycling materials). Currently, measurements of fungal exposure rely on air sampling for culturable fungi or total spore counts. Although sampling and testing for mycotoxins and fungal surrogates, such as ergosterol and glucan, are possible, the application has not been widely used.

In addition to air sampling, assessing indoor fungal contamination requires careful review of the building history and visual inspection by an experienced environmental professional. Suspected contamination should be collected by source sampling and confirmed to be fungal growth. This approach not only identifies the sources of contamination but also facilitates eventual removal and control of fungal growth.

During an investigation for fungal contamination, both building structures and furnishings, and the HVAC system should be inspected. The previous issue of this newsletter discussed biological contamination (including fungi) of the HVAC system. This issue addresses the investigation of building structures and furnishings.

Air Sampling

When planning for air sampling, one needs to consider the following: which sampler has better collection efficiency, what media to use, when and how long to sample, how many samples to take, what to compare to, which microbiology laboratory to use, and what to do with the results.

A number of air samplers for culturable fungi and for total spore counts are commercially available. These samplers are widely used by allergists, industrial hygienists, and environmental professionals. Sampling for culturable fungi includes passive techniques, such as the settling method, and volumetric samplers. The settling method is often employed by practicing allergists for their patients. This method favors larger spores and its usefulness is limited. There are many volumetric samplers available. Three commonly used ones are discussed in the following paragraph. There are also air samplers for total spore counts. Sampling for culturable fungi yields results reflecting the identities and viability of spores. It may take one to two weeks for analysis. Results of total spore counts may be available in hours or in days. However, the viability of spores is not known and the identities of spores are presumptive at best.

The volumetric samplers for culturable fungi are the Andersen single stage N-6 impactor (Andersen N-6), the Reuter Centrifugal Sampler, and the Surface Air System sampler. Other models of these samplers are also available. Andersen N-6 is popular with industrial hygienists. Approximately 75% of air samples analyzed in our laboratory are taken with the Andersen N-6 sampler. Other samplers account for 25% of the samples. Comparisons of these samplers are available in several references (see Reference 2). These samplers require a device containing nutrient media to receive fungal spores and allow them to grow into colonies after incubation. Many different fungal media are available. For general fungal population, 2% malt extract agar (MEA), cornmeal agar (CMA) and rose bengal agar are often used. For specific fungi, such as *Stachybotrys chartarum* (= *S. atra*), CMA or Czapek cellulose agar (CCA) are preferred. Other fungal media may be used if they are available.

One should keep in mind that the three samplers collect "grab samples" usually for less than fifteen minutes (more often 1-8 minutes). When taking into consideration the spatial and temporal variability of airborne fungi, sufficient samples are necessary to draw a complete picture of fungi in the air. Conclusions should not be made based on only a few air samples.

For total spore counts, spores are collected either on a membrane filter or on grease-coated slides. Filter cassettes powered with industrial hygiene pumps can be used. The Burkard spore trap (available from the manufacturer in England or from re-sellers in the U.S.) and Allergenco samplers collect spores and particles onto a grease-coated 1" x 3" glass slide. The recently introduced Air-O-Cell cassettes are also popular with industrial hygienists. These sampling methods are not suitable for collecting bacterial particles.

There are many other air samplers introduced over the last few years, however, it is my opinion that one should select air sampling equipment that has been scientifically validated or compared with known existing samplers with good performance. A new sampler or sampling device may not be a better mouse trap.

Source Sampling

Fungi do not grow and reproduce in the air. They originate on surfaces or in substrates. Many building materials, such as ceiling tiles, wallboard, wallpaper, wood, and soiled fibrousglass insulation, are well known substrates for fungal growth when they become wet or retain moisture. Water stain, discoloration, and mildew or sooty growth are signs of fungal growth. Wipe samples taken with sterile swabs or bulk samples of the materials should be collected for analysis. Reference samples from "clean" surfaces or of similar materials may be collected for comparison. Elevated fungal levels dominated by one or two fungal types are often indications of fungal amplification.

To quickly confirm fungal growth on surfaces, a clear sticky tape can be used to remove "growth." The tape is then examined under a compound microscope by an experienced mycologist. This procedure provides qualitative, descriptive results. Identifications of fungi are possible if sufficient fungal characters are available on the tape samples. This method is generally not suitable for bacterial sampling because bacterial cells are too small for direct microscopic examination.

Soiled carpets and fibrous glass insulation materials may be vacuumed with a filter cassette and a vacuum source (pump). Dust collected in the cassette can be weighed, extracted and inoculated onto appropriate media for growth. Dust samples collected with this method can also be analyzed for various biological contaminants, such as bacteria, endotoxin, and allergens.

Sample Analysis

When selecting a microbiology laboratory, find one that has a mycologist with an advanced degree on staff. A good mycologist not only identifies fungi but also has knowledge of the possible sources of various fungi, their health implications, and effective control measures. Air samples for environmental fungi are generally incubated at room temperature (between 20-28°C). However, if *Aspergillus fumigatus* and other thermotolerant fungi are the main interest of sampling, the incubation temperature should be 35-37°C. Enumeration and identification are usually done in seven to ten days. Currently, most laboratories identify fungi to genus levels or to species for *Aspergillus* and *Stachybotrys* species.

For wipes and bulks, samples are extracted, diluted serially, and inoculated onto appropriate fungal media. No direct inoculation should be allowed because environmental samples often include a mixture of fungi and bacteria. All samples should be incubated at the appropriate temperature range. For fungi in general, MEA and an additional medium should be used. MEA is the medium used by most mycologists to study and describe fungi in culture. For slow growing fungi, such as *Stachybotrys chartarum*, a low nutrient medium, such as CMA or CCA, provides a better chance of recovery. For xerophilic fungi (fungi growing at low water activity or low water content), DG18 is the medium of choice. Many indoor fungal contaminants, including species of *Aspergillus* and *Wallemia sebi*, are xerophilic.

Samples taken for total spore counts using a membrane filter can be cleared, stained with biological dyes, and examined and counted under a compound microscope at a magnification of 400X or higher. Samples taken with grease-coated slides can be stained and examined directly.

Interpretation of Results

Because there are no generally accepted guidelines or standards for fungal bioaerosols, comparative sampling becomes necessary. Usually outdoor samples and samples taken from non-problem locations are collected as a reference for comparison. A few proposed guidelines for fungi have been published, however, they should be used with care and only

for screening purposes but not as a health standard. These guidelines may also be considered as "performance standards." The importance of reference samples is such that a significant number should be collected for each job.

For practical purposes, comparisons with reference samples are the most useful approach. In general, indoor fungal concentrations should be similar to or lower than outdoors. Residential constructions are usually leaky and tend to have fungal levels similar to outdoors. Indoor fungal types should also be similar to outdoors. If fungi at a significant level are only found indoors, this often suggests indoor amplification of the fungi. Furthermore, the detection of some fungi even at low levels in the air may require further evaluation. Any presence of slimy-spored toxigenic fungi, such as *Stachybotrys chartarum* and *Acremonium strictum*, may suggest an indoor contamination source. The consistent detection of some fungi, such as species of *Aspergillus*, *Paecilomyces*, *Penicillium*, *Trichoderma*, and *Wallemia* could indicate water damage and fungal amplification.

Results of total spore counts often have a limited use because they usually have low detection sensitivity and wide standard deviations and the spores can only be presumptively identified. In practice, total spore counts are used to estimate airborne spore loading in a heavily contaminated environment. However, the total spore count method may be a better assessment tool for airborne fungal spores than culturable fungal sampling if spore counting and quantification procedures can be standardized and standard deviations can be reduced.

Results of wipe and bulk samples give strong indications as to whether a sample is contaminated or not. A contaminated sample often results in a pure culture or a mixture of no more than two to three dominant fungi. A high fungal level dominated by one or two fungi is suggestive of fungal amplification in situ. Contaminated materials or surfaces can then be removed or disinfected.

Prevention, Remediation, and Control

Fungi do not grow well indoors if there is insufficient water and moisture in materials and substrates. The control of moisture, water, and excessive humidity is the most important factor in controlling fungal growth indoors.

If fungal growth is detected in the indoor environment, remediation and control procedures should consider the following factors: 1) determination of fundamental problem (water and moisture) leading to their growth, 2) minimizing spread of fungal propagules, 3) method of removing and/or treating fungal growth; and 4) determining whether biocides should be used or not. If the first factor cannot be determined and fixed, an expensive remediation program is only a temporary fix. Fungi will grow back in no time.

The current recommended procedures in remediating and controlling indoor fungal growth are: 1) stop and control all moisture and water problems; 2) remove contaminated materials under containment to avoid dispersal of fungal spores; 3) HEPA vacuum fine dusts and particles; 4) topical use of biocides only if it is absolutely necessary; 5) clearance inspection, sampling, and testing to assure a complete removal of contamination; and finally, 6) placing the environment under strict moisture control for a period of time. Quick fixes, such as application of bleach solutions, only temporarily remove surface growth. Fungi will grow back in one to two weeks if excessive moisture and water persist. Furthermore, dead fungal spores may still contain allergens and toxins.

Final Comments

Many mycologists, microbiologists, and health professionals actively involved in dealing with fungal contamination in buildings have the consensus that people should not be allowed to live or work in a fungi infested environment. Environmental professionals should carefully make observations of possible fungal contamination in a building and its associated heating, ventilating, and air-conditioning systems. A comprehensive sampling should include air sampling as well as source sampling. Reference samples should always be included in any sampling.

The results of air sampling provide an estimate of possible human exposure and an indication of possible indoor contamination. However, air samples may give false negative results. Only a thorough inspection and analytical confirmation can ascertain whether there is fungal contamination in a building.

There is currently no governmental licensing program for environmental microbiology laboratories. The American Industrial Hygiene Association started an Environmental Microbiology Proficiency Analytical Testing (EMPAT) program in 1996. Subsequently, an Environmental Microbiology Laboratory Accreditation (EMLAC) program was initiated in January 1999. Even with these programs, it is still difficult to determine whether a laboratory is truly competent in analyzing environmental samples for fungal components or not. A mycologist with good qualifications (such as a minimum of a master degree from a University with an established mycology program) on staff is the single most important factor.

A preventive program with procedures for proper moisture control, quick response to water intrusion, and clean and hygienic maintenance of a building and its associated HVAC systems will minimize the occurrence of fungal growth and save money in the long term. If a building has fungal growth, a comprehensive program for remediation, control, and prevention should be used to remove the growth and prevent its reoccurrence. An incomplete program may cause secondary contamination or allow fungi to regenerate and re-colonize the environment.

References:

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