

ASSESSMENT OF 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)- β -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. In addition, information should be gathered and samples may be taken and analyzed for endotoxins, allergens, and other bioaerosols to determine whether each individual bioaerosols may play a role in the health manifestation or not. A physician, whether a board certified occupational health physician, an immunologist, an allergist, or a pulmonary specialist, may be involved in the determination of etiology agents responsible for human symptoms.

During an investigation for fungal contamination, both building structures and furnishings, and the HVAC system should be inspected. A history of the building should be told.

Personnel

Depending upon the nature of a case, a team of experts with various expertise is often required to build a case. The expertise required may include medical, structural and engineering, environmental, or mycological and biological. Additional professionals and experts (such as remediation and construction experts) may be added to the list when there is a need depending on the case. This section will discuss the expertise of each expert professional. Each attorney should conduct his/her due diligence to thoroughly investigate, evaluate and review individual expert's educational background, competency, professionalism, integrity, and experience. This is particularly important in light of the fact that there are no recognized or government sponsored certification and license program for various professions for dealing with mould issues.

1. Medical professionals: If personal injury or health related issues are a part of the case, physicians must be included in the team. Specialty physicians, such as Board Certified Occupational Physicians, allergists, immunologists, pulmonary specialists, infectious disease specialists, or epidemiologists, are some of the medical expertise considered. Because health effects due to mould exposure are relatively obscure until recently, most physicians have little experience with making diagnosis of mould exposure. Select physicians who are open mind and diligent in conducting review of medical literature and research. In addition to examining patients, they may provide assistance to environmental professionals on determining sampling and testing, and review medical opinions offered by opposing medical professionals.
2. Structural and engineering professionals: These professionals may include architects, civil engineers, mechanical engineers, structural engineers, building engineers, to roof specialists. Although an architect or an engineer with a professional license (such as AIA or PE) is good to have, it does not necessarily guarantee the best competent professional available. Both architects and engineering professionals are useful in finding and determining structural and engineering problems and flaws of a building leading to mould growth and infestation. They may also be asked to provide solutions to the structural problems and design a mould remediation project.
3. Environmental professionals: These professionals may include environmental consultants or Certified Industrial Hygienists (CIH's). They may be asked to review: the facts in the case; perform an independent assessment of the building in question; render an opinion on the likelihood of the cause of the mould and the likelihood of health effects; and offer specifications to remediate the property and restore it to its previous condition. Although many environmental

consultants are very competent in providing the services, a Certified Industrial Hygienist is often preferred. A CIH is a widely recognized professional certification, including governmental agencies. However, a CIH may perform ergonomics, asbestos and lead assessments, hazardous site assessment, to indoor air quality and mold assessment. It is recommended that potential candidates, whether an environmental consultant or a CIH, should be interviewed and evaluated for their competence and experience in mold assessment.

4. **Mycology and biology professionals:** Mycology is such a unique branch science of microbiology and biology that only mycologist has a true understanding of the science. The scientists who study fungi or mycology are called mycologists. There are not many colleges and universities offering mycology program. Mycology program is often offered as a Ph.D. graduate degree program. Therefore, they are not many mycologists around. Furthermore, very few mycologists have expertise in the group of mould and fungi, which are found in water-damaged moldy environments. For example, there are mycologists who specialize in wild mushrooms, which are only infrequently found growing indoors. It is Important to find the right expertise. Mycologists may offer information on various scientific aspects of mould and fungi as well as guidance on their identification, elimination and control. In few occasions, biologists with various expertise may be call upon to address issues caused by other biological agents, such as dust mites, cockroaches, pollens, insects, etc.
5. **Other professionals:** Biochemists who specialize in mycotoxins or other chemical toxicants may be called on as an expert. Toxicologists who specialize in toxic effects of mycotoxins and other chemical toxicants may be useful in certain case situations. However, there are very few true experts in these areas. Careful review of such experts is critically important.

The Science of Mycology

Mycology is the scientific study of fungi (The Dictionary of the Fungi, 2001) or the branch of biology dealing with fungi (Webster's College Dictionary). Fungi are heterotrophic eukaryotes (with true nucleus) producing exoenzymes and absorbing their nutrients by a network of hyphae and reproducing through development of spores and belong to the Kingdom Eumycota (Kingdom of Fungi) or the Kingdom Chromista (Kendrick, 2000). Eukaryotic form of organisms is considered much more advance than the prokaryotes, i.e. bacteria. The genetic materials (DNA) are organized into chromosomes and in a nucleus. In addition, the cellular structures are often highly compartmentalized. Most fungi are free-living, and they may live as saprobes, parasites or symbionts. A few fungi are parasitic or opportunistic pathogens of animals and humans. Most fungi found growing in indoor environments are saprobes, weak plant pathogens, or opportunistic pathogens. A few fungi (such as *Aspergillus fumigatus*) are important opportunistic pathogens in hospitals or health care facilities.

Fungi known to produce a wide variety of secondary metabolites, including poisons, mycotoxins, and antibiotics. Mycotoxin-producing fungi are conveniently called toxigenic fungi. A recent review suggests that fungal species in 46 genera are capable of producing mycotoxins. The major fungal genera including species that produce mycotoxins are *Aspergillus*, *Fusarium*, *Gliocladium*, *Memnoniella*, *Penicillium*, *Stachybotrys*, *Trichoderma*, etc. It is important to note that the majority of fungi have not been screened for their mycotoxin-producing capability (Kendrick, 2000). Some secondary metabolites may be used to human's benefit. Penicillin, cephalosporins, and cyclosporines that are derived from fungi are used extensively in treating bacterial infections or in bone marrow or organ transplant patients.

The Kingdom Fungi includes four phyla: Chytribiomycota, Zygomycotina, Ascomycotina, and Basidiomycotina. Another group deutermycotina is not considered a formal taxonomic category because it often includes asexual phase of mainly ascomycetes and some basidiomycetes. Unfortunately, most moulds and fungi identified growing in the indoor environment are in deutermycotina and, to a lesser degree, in Zygomycota (such as *Rhizopus stolonifer*, *Mucor plumbeus*), Ascomycota (such as *Chaetomium globosum*), or Basidiomycota (such as wood decay fungi).

The scientists who study fungi or mycology are called mycologists. In most cases, credible mycologists are likely to have a Ph.D. degree and a track record of publishing in peer-review journals and scientific literature. There are only a handful of Colleges and Universities in the US offering mycology graduate degree programs, usually in a biology, botany, or plant pathology department. Some mycologists focus their study on a single fungus or on a small group of fungi. However, there are also mycologists who have a very broad interest in many different groups of fungi. An ideal mycologist for assisting in an indoor mould contamination issue should have a broad training and interest in many different groups of fungi as well as their biology.

Fungi do not contain chlorophyll and, therefore, cannot synthesize carbohydrate and sugars. They must obtain nutrients by absorption from the surroundings. Their cell walls contain chitin and glucans. Many fungi are capable of producing cellulases to breakdown cellulose into simple sugars. Many of these fungi are commonly found on water damaged building materials because of such capability. Some known cellulolytic fungi that may be found indoors are *Trichoderma* species, *Stachybotrys chartarum*, *Chrysosporium pannorum*, *Oidiodendron griseum*, *O. cerealis*, *Gliomastix murorum*, *emmoniella echinata*, etc.

Some fungi that grow into colonies with a well-marked mycelium or spore mass are called moulds. This is in contrast to some unicellular fungi, such as yeasts. Some fungi are diphasic. They may produce filamentous mould phase, yeast phase, or both, depending upon temperature, nutrients, etc.

Information Gathering and Assessment

Although there are many requirements for moulds to grow, the primary reason is moisture. It is important for a consultant to look for moisture sources as well as the pathways of moisture migration inside a building. Outdoor moisture may come into a building as water through leaks, cracks, or floods, or as humidity through building envelope and structures, doors and windows, or air intakes. Moulds are likely to grow if moisture intrusion is not taken care of on a timely fashion. The first order of the process is to determine the routes and pathways of moisture intrusion. This is followed by visual inspection and identification of mould growth, sampling and testing for moulds, and, finally, defining the extent of mould growth and contamination. Because moulds produce spores for dispersal and dissemination, the entire indoor environment is considered contaminated even though mould growth may be observed only in part of the environment. When planning for sampling and testing, biological contaminants other than moulds (such as endotoxins, pollens, dust mite allergens, cat and dog allergens, cockroach allergens) should also be taken into consideration to rule in or rule out their implication.

In an assessment and investigation of indoor microbial contamination (Note: this often means fungal and/or bacterial contamination in the indoor environment), there are many reasons that field samples are collected and analyzed. It is important that industrial hygienists and environmental consultants know why, how, and what to sample and to test for. It is also very important that they know how to **properly and correctly** interpret the laboratory results. Sampling, testing and interpreting the results are parts of the assessment process and should be used in correlation with the environmental data and field observations.

There are no governmental and professional protocols for sampling and testing for microbes (except for certain waterborne microbes) at this time. Traditional sampling and analytical procedures are usually used and acceptable. Currently methods used in microbial analyses are conventional or modified methods used by microbiologists. These methods are acceptable by the microbiology community. However, several “new” sampling and analytical procedures have been “invented” by entrepreneurs since the rush into mold and microbial sampling and testing began approximately five years ago. These “new” procedures are often not scientifically evaluated and validated. Users of such procedures must be aware of the pitfalls. Furthermore, there are no numeric standards (such as PEL or TLV) for airborne or surface fungi and microbes indoors. Result interpretation is based on comparison of outdoor background or indoor references.

Sampling & Testing

1. When planning sampling and testing for mold consider the following:
2. **Selection of an environmental microbiology laboratory**
3. **Selection of analysts:** polymerase chain reaction (PCR), fungal spores, culturable fungi and bacteria, fungal confirmation and identification based on optical microscopy, mycotoxins, fungal biomass (glucans, ergosterols), endotoxins, pollens, microbial volatile organic chemicals (VOC's), and allergens.
4. **Selection of sampling methods**
5. **Sensitivity of the tests**
6. **Field sampling quality control and quality assurance**
7. **Reporting and laboratory support**

1. **Selection of an environmental microbiology laboratory:** There is currently no governmental certification and licensing requirement for an environmental microbiology laboratory, or more specifically a mycology laboratory. The American Industrial Hygiene Association (AIHA) provides an environmental microbiology proficiency analytical testing program (EMPAT) as well as an accreditation program for environmental microbiology (Environmental Microbiology Laboratory Accreditation Program – EMLAP). The EMPAT is a volunteer program. Therefore, the participation of EMPAT does not guarantee the quality of the laboratory. The accreditation program requires that a laboratory achieves an average score of 85% or higher in EMPAT and meets all QA/QC requirements established by AIHA. A laboratory accredited under the program means it meets the basic standards. Mycology and bacteriology are covered in the program. However, the program does not specifically require a laboratory be supervised by a mycologist for its mycology testing or a bacteriologist for the bacteriological testing. The laboratory must also have a good collection of mycology and bacteriology references and literature. If analysis for fungal and bacterial byproducts (such as glucans, ergosterols, or endotoxins), VOC's or mycotoxins is required, a laboratory with demonstrated expertise in such analysis is necessary. Users of the laboratory services must do their due diligence to make sure that a laboratory of their choice has a qualified staff.
2. **Selection of analyses:** There are many analyses available for detecting fungal spores, fungi, bacteria, and their byproducts in samples collected from indoor environments. Each analyst may provide certain useful information and purposes. Some of the commonly used tests are discussed below.
 - A. **Quantitative polymerase chain reaction (QPCR):** This new advanced technology is based on species-specific fungal DNA primers identified and developed at the research laboratories (including laboratories of the Centers for Disease Control and Prevention and the U.S. Environmental Protection Agency in Cincinnati, Ohio). The technology is based on the science that species-specific DNA sequences can be amplified *in vitro* and detected for fungal and bacterial identification and quantitation. It can be applied to environmental air, bulk, dust, wipe, or bulk liquid samples. It is highly sensitive. The detection sensitivity is much higher than any of the analyses discussed below. A rapid turnaround time of 24-48 hours is likely. The precision and accuracy of the test is very high if the analysis is performed under the supervision of a qualified and competent scientist, usually with a Ph.D. degree.
 - B. **Spore counts:** This analysis is based on microscopic identification and enumeration of fungal spores and is only feasible for spore-trap air samples (although misuse of this method on tape lift sample is common). Appropriate fungal identification is based on colony morphology, spore production and formation, various macro- and microscopic characters, in addition to spore. The identification based on spore alone is presumptive at best and the viability of spores is unknown. More significantly, there is no standard or generally accepted analytical method. This is considered a screening tool. However, in a mold remediation situation spore count analysis provides a quick turnaround for the determination whether the remediation is cleared or not. This analysis is not suitable or useful in samples collected from a dusty environment because of dust interference in the analysis.
 - C. **Culturable fungi:** This analysis generally requires an incubation period of at least seven days on appropriate nutrient media and incubation temperature ranges. Samples can be air, wipe, bulk, dust, or liquid. For general purposes, incubation at near 25°C for at least seven days is suggested. If samples are collected from health care facilities and opportunistic fungal pathogens (such as *Aspergillus fumigatus*) are of concerns, samples should be incubated at 35-37°C for two days or longer. Fungal nutrient media may be for general isolation or for selective isolation. General-purpose media include malt extract agar (MEA), cornmeal agar (CMA), rose bengal agar (RBA), oatmeal agar (OA), potato dextrose agar (PDA), etc. Malt extract agar, CMA, and OA are sometimes used in the description of fungi. Cornmeal agar was used in describing species of *Stachybotrys* and *Memnoniella* in culture (Jong and Davis, 1976). Malt extract agar is important in studies of *Aspergillus* and *Penicillium* in culture (Pitt, 2000; Klich and Pitt, 1988) and is a common medium used by mycologists. Therefore, they should be used in identification and speciation of certain fungi. Selective media include DG18 agar, MEA plus 20% or 40% sucrose, cellulose agar and others. DG18 agar and MEA plus 20% or 40% sucrose are for selecting xerophilic fungi. Cellulose agar is for selecting and testing cellulolytic fungi, such as *Stachybotrys chartarum*, *Trichoderma* spp., *Chrysosporium pannorum*. Industrial hygienists and environmental consultants may want to discuss the selection of media and incubation temperature with a knowledgeable mycologist.

- D. **Culturable bacteria:** This analysis generally requires an incubation of samples on appropriate nutrient media and at incubation temperature ranges. Samples can be air, wipe, bulk, dust, or liquid. For general environmental bacteria, incubation at or near 25°C is suggested. If samples are collected from health care facilities and opportunistic bacterial pathogens (such as *Legionella pneumophila* or *Pseudomonas aeruginosa*) are of concerns, samples should be incubated at 35-37°C. Bacterial nutrient media may be for general isolation or for selective isolation. General-purpose media include tryptic soy agar (TSA), blood agar (BA), nutrient agar, etc. Selective media are often used for the selection of specific bacterial species. For example, buffered charcoal yeast extract agar (BCYE) and BCYE with antibiotics are used to the selective isolation of *Legionella* species. Other selective procedures, such as acid treatment or heat shock treatment, may be used for the selection of specific bacteria.
- E. **Fungal confirmation and identification based on optical microscopy:** Bulk or tape lift samples taken from visible, suspect “fungal growth” is analyzed microscopically. This analysis is to confirm the observed suspect fungal growth and to identify observed fungi. This is a commonly used procedure in a mold assessment. However, many consultants and laboratories have misapplied this procedure for detecting and counting fungal spores from a surface with no sign of fungal growth.
- F. **Mycotoxins:** Testing for mycotoxins in environmental samples may be important because the identification of a “toxigenic” fungus is not necessary proving the presence of mycotoxins. Environmental air, dust, or bulk samples can be analyzed for mycotoxins using High Pressure Liquid Chromatography (HPLC), Gas Chromatography/Mass Spectrometry (GC/MS), or Liquid Chromatography/Mass Spectrometry (LC/MS). The analysis of mycotoxins is highly sophisticated. The selected laboratory must be supervised by a reputable scientist with experience in mycotoxin research or analysis.
- G. **Endotoxins:** Endotoxin (primarily lipopolysaccharide) is a chemical component of gram-negative bacterial cell walls. In addition, it is a known bio-active agent associated with fever, flu-like symptoms, and other respiratory illness. Sampling and testing for endotoxin can be used to assess gram-negative bacterial biomass or to determine exposure to this bio-active agent when there is a diagnosis of respiratory diseases involving fever, flu-like symptoms. Environmental air and dust samples may be collected and analyzed using the endotoxin-specific LAL assay. There are several variations of LAL assay. The most accurate and reproducible method is kinetic chromogenic method. This is also the method of choice if endotoxin testing is necessary.
- H. **Fungal glucan:** Glucan (primarily β -1,3 glucan) is a chemical component of fungal cell walls. In addition, it is a known bio-active agent associated with inflammation and scarring of lung tissue. Sampling and testing for glucan can be used to assess fungal biomass or to determine exposure to this bio-active agent when there is a diagnosis of lung diseases involving inflammation and scarring of the lung tissue. Environmental air and dust samples may be collected and analyzed using the glucan-specific LAL assay.
- I. **Fungal ergosterol:** Ergosterol is a chemical component of fungal cell membrane. Sampling and testing for glucan can be used to assess fungal biomass. This analysis is seldom used in a fungal assessment.
- J. **Fungal, bacterial, or microbial volatile organic chemicals (VOC's):** Fungi and bacteria produce a wide variety of volatile organic chemicals during their growth. Some of the VOC's are odorous and responsible for the “musty, moldy” odor. In addition, the VOC's are considered respiratory irritants. Environmental air samples can be collected and analyzed for VOC's using GC/MS.
- K. **Allergens:** Although allergens, such as dust mites, dog, cat, or cockroach, are not microbial, they are discussed because they, like fungal spores, can produce allergic symptoms. Commercially available analysis for these allergens is based on enzyme-linked immunosorbent assays (ELISA).
- L. **Pollens:** Although pollens are most likely of outdoor origin, they are common in indoor air or dusts. They may infiltrate the indoor environment with air current through doors, windows, or outdoor air intakes. In addition to

their allergenicity, the presence of pollen can signal poor filtration of the outdoor air in the HVAC system. Furthermore, an accumulation of pollen may serve as nutrient for fungal and bacterial growth when wet. The analysis of pollen is by microscopy.

3. Selection of sampling methods: The most important consideration in selecting a sampling method is collection efficiency. In addition, take into consideration several issues: sample media, flow rates and sampling duration, sample preservation, sample packaging and shipping. Environmental consultants and field personnel must consult with a qualified and competent laboratory for the information of sample media, flow rates and sampling duration, sample preservation, sample packaging and shipping. For air sampling for PCR analysis, clean filter cassettes and air sampling pumps are used. For spore trapping and counting, sampling equipment, such as Burkard and Allergenco samplers, are commercially available. The personal Burkard is the grandfather of spore trapping sampler and ideal for sampling indoors. Several spore-trap cassettes have been devised and available commercially. However, collection efficiency and validation of these devices require further scientific evaluation. The spore trapping method is also used for collecting airborne pollens.

Several air samplers for **culturable fungi and bacteria** are available commercially. The most common one is the Andersen N-6 single stage sampler. Andersen samplers also have a six-stage and a two-stage model. The sampling mechanism is based on impaction of spore in the air stream onto agar surface. After incubation, fungal colonies are counted and identified. Other samplers for culturable fungi and bacteria include Spiral Air System (SAS), Microbios, Biotest, a clone of Andersen N-6 sampler, and others. Airborne fungal spores may also be collected onto a filter cassette and then cultured onto agar plates. However, the filter cassette method is not appropriate for airborne bacteria because of desiccation effects to bacterial cells.

Bulk material samples or tape lift samples can be collected for microscopic examination of **fungi**. Clear cellophane tape is ideal and commonly used for this purpose.

Air and dust samples for **mycotoxin** testing are collected onto filter cassettes. If air sample is collected, a very large air volume is necessary to achieve a good quantitation/detection limit.

Air and dust samples for **endotoxin** testing are collected onto specialty filter cassettes. Because endotoxin is very common in the nature, endotoxin (or pyrogen) free filter cassettes must be prepared to minimize background contamination.

Air and dust samples for **glucan** testing are collected onto specialty filter cassettes. Because glucan is very common in natural substances, specialty filter cassettes must be prepared to minimize background contamination.

Air and dust samples for **ergosterol** testing are collected onto specialty filter cassettes to minimize background contamination.

Air samples for **microbial VOC's** are collected into an air collection bag or into collection tubes packed with various absorbents. Each laboratory may formulate its own combination to achieve maximum collection. Discuss the choice of collection device with the laboratory.

Dust samples for **allergen** testing are collected onto filter cassettes or interceptor bags. If air sample is collected, a very large air volume collected in a filter cassette is necessary to achieve a good quantitation/detection limit.

4. Sensitivity of the tests: When sampling and testing for fungi or bacteria, the sensitivity includes both qualitative sensitivity and quantitative sensitivity. Qualitative sensitivity is defined as the accuracy of fungal, bacterial, or spore identification. This heavily relies on individual analyst's education background, training and experience in mycology and bacteriology, continuing education, and laboratory's quality control program. Quantitative sensitivity depends on sampling equipment's collection efficiency, sample air volume (if it is an air sample) or sample quantity, sample processing in the laboratory, analyst's training and experience, and laboratory quality assurance and quality control program (QA/QC).

Samples for mycotoxins, glucan, ergosterol, and microbial VOC's are analyzed using either bioassay or chemical analysis. Sensitivity of the tests depends on equipment's collection efficiency, sample air volume (if it is an air sample) or sample quantity, sample processing in the laboratory, sensitivity of the test equipment, and laboratory quality assurance and quality control program (QA/QC).

5. Field sampling quality control and quality assurance: Laboratory test results are only as good as the quality of the samples analyzed. Although laboratory QA/QC is very important, field sampling QA/QC is often overlooked. A poorly collected sample or a contaminated sample is not likely to yield accurate results even if a laboratory provides the best performance. Field sampling QA/QC may include all or some of the followings: calibration or validation of air sampling equipment, checking for cleanness of sample media, the use of aseptic technique, following established or appropriate sampling protocol, shipping and packaging of samples, speedy delivery of samples to the laboratory of choice, and a complete documentation and chain of custody.
6. Reporting and laboratory support: Laboratory reports should include all important and relevant information of the samples and the test. The information is not only essential for the person who receives it but also third parties, who may be asked to review the report. In addition, a good qualified and competent laboratory should also provide biological, ecological, toxicological, and other information regarding the identified fungi and fungal chemical byproducts which are important and relevant to the users of the report.

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 resellers 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 fibrous glass insulation, are well known substrates for fungal growth when they become wet or retain moisture. Water stain, discoloration, and mildew or sooty growths 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.

Sampling 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, EA 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.

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