

Chapter 11

Sampling and Analysis for Mold Contamination in the Indoor Environment

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Synopsis

11.1 Introduction

11.2 Planning for the Monitoring of an Indoor Environment

11.3 Methods for Sampling and Analysis

A. Analysis

B. Air sampling

C. Source/surface sampling

D. Interpretation of Data

References

11.1 Introduction

Fungi are ubiquitous and contribute to the decomposition of organic material and the natural recycling of nutrients from wood, wood-products, and detritus in the environment. They are microscopic organisms that form visible colonies or other structures when growing on a suitable substrate under favorable environmental conditions. Filamentous fungi, also termed molds, reproduce through the production of microscopic spores, many of which are dispersed by wind, rainfall, and physical disturbance. Mold spores are generally present in indoor environments as mixtures of fungal genera with no one organism predominating. However, molds can colonize and grow in indoor environments that have been subjected to water accumulation or water damage resulting in elevated concentrations or the predominance of some species that may cause in adverse human health effects. Monitoring in indoor environments is performed to determine if unusual fungal populations or concentrations are present. Monitoring is also performed following remediation of mold-contaminated indoor environments to verify the efficacy of the remediation. This chapter

focuses on currently used methods for sampling and analysis of air and surfaces in office, residential, and classroom environments.

11.2 Planning for the Monitoring of an Indoor Environment

Prior to collecting samples in an indoor environment, a plan should be prepared that outlines the reasons for the monitoring and the goals of the investigation. This planning is needed for the selection of meaningful sampling and analysis methods. There are four main reasons to conduct an on-site investigation for mold contamination in a building. The first three are focused on the hypothesis that contaminant mold(s) may be present in the indoor environment. The fourth reason is to verify clean up of a mold-contaminated environment. The first reason for an investigation is in response to complaints from employees or occupants of a building that indoor air quality is poor or the belief that fungal contaminants are causing adverse indoor environmental quality. The second is in response to a physician reporting that a patient's symptoms are consistent with exposure to mold. The third reason, evidence or suspicion of water damage indoors, is the most compelling. A sudden incidence of water damage such as flooding, plumbing overflow or backup, or structural damage during a rain storm are among the obvious water damage events that prompt indoor surveys for mold indoors, but insidious water damage resulting from hidden plumbing problems, roof leaks, condensation, or construction defects is often not found until the survey has identified the presence of mold contamination. The fourth reason is to verify that remediation of a mold-contaminated environment has been effective. Regardless of the reason for the investigation, an on-site investigation should be conducted to visually assess the physical parameters of the building and to obtain information from the building occupants. On-site inspections in commercial buildings and schools can also provide a forum for discussions with building operators and maintenance staff. The on-site investigation conducted in post-remediation monitoring is also an opportunity for discussions with the remediation contractor. Once a decision to conduct an investigation has been made, it is important to determine the goals of the monitoring so that the proper types of data can be obtained. The types of data that are usually desired in the investigation of indoor environments are:

1. documentation of the presence of moisture accumulation/water damage conditions that are likely to lead to mold contamination;
2. documentation of visible contamination on surfaces; and
3. quantitative and qualitative measurements of the concentration and composition of airborne and surface-associated fungal populations; or the presence of fungal allergens, toxins or other by-products of fungal metabolism that indicate fungal growth (e.g., ergosterol, β -1,3 glucan).

The documentation of moisture intrusion or accumulation is provided through visual assessment of water-damaged areas and by using commercially available moisture meters for surrounding areas that do not appear to have overt water accumulation. In addition, the collection of a photographic record of surfaces is useful to document the presence of visible contamination. However, quantitative and qualitative measurements of the populations of mold present require the sampling and analysis of the air and of surfaces. Selection of methods to be used in the monitoring must include considerations of the performance evaluation of the methods, the number of samples to be collected, the locations of sample sites, the cost of monitoring, and the types of data that are needed. The expertise of the sampling team and the analytical laboratory is also an important consideration.

11.3 Methods for Sampling and Analysis

Monitoring of air and surfaces requires consideration of both sampling and analysis techniques, with the selection of a sample collection method dependent on the analytical method(s) to be used. It is important to note that consultation with the environmental laboratory that will be responsible for the analysis is critical before conducting an investigation.

A. Analysis

Samples for airborne and surface-associated fungi have traditionally been analyzed by culture and/or by microscopic examination (ACGIH, 1999; Buttner *et al.*, 2002a). Determination of fungal allergens, ergosterol and β -1,3 glucan indoors is not routinely conducted in indoor monitoring, but this may be useful in the future for exposure assessment.

1. Culture analysis

Culturing involves growing the mold on laboratory culture media and counting the resulting colonies. Selection of the media used to grow the colonies is critical when monitoring for culturable mold. Malt extract agar is a broad-spectrum growth medium commonly used for culture of mold in indoor environments (ACGIH, 1999), but a variety of other agar media has also been recommended. It is important to remember that no single growth medium will support the growth of all fungi. A minimal medium with glycerol has been suggested to increase the likelihood of culturing xerophilic fungi (Pasanen, 1992; Smid *et al.*, 1989; Verhoeff *et al.*, 1990) and antibiotic-amended media are useful in inhibiting the growth of bacteria that may prevent or obscure the culturing of slower growing fungi.

In general, samples for culture analysis are inoculated onto agar and incubated at room temperature (23-25°C) for five to ten days with daily inspection and analysis. Incubating additional agar plates at elevated temperatures permits the selective isolation of some fungi (e.g., *Aspergillus fumigatus*) that grow optimally at high temperatures.

Data are reported as the number of colony forming units (CFU) with the assumption that each colony arose from a single fungal spore or fragment of vegetative hyphae. Air samples collected using a volumetric air sampler result in data that are reported as CFU per cubic meter of air (CFU/m³). Surface sample data are reported as the number of CFU/gram of sample collected or as the number of CFU/area (e.g., CFU/m² or CFU/cm²).

2. Microscopic analysis

Microscopic analysis provides information on the total fungal population in a sample, both culturable and non-culturable. Fungal contaminants that may not readily grow in culture (e.g., basidiospores) or those that are difficult to grow can be detected as this method does not require the formation of colonies on an agar surface. Microscopic analysis is usually performed using standard light microscopy while other microscopic methods, such as interference, phase contrast, and scanning electron microscopy, are mostly limited to research settings.

Air samples for light microscopic analysis are collected by impaction of particles directly onto a glass slide coated with a mounting medium. Surface samples are collected by pressing surfaces with transparent tape. These samples are often referred to as "tape lift samples." Samples col-

lected for microscopic analysis are generally treated with a stain (e.g., lactophenol cotton blue or acid fuchsin) to enhance examination of fungal structures and enumeration of spores. Some laboratories scan the entire slide at low magnification and then analyze only a fraction of the slide under higher magnification. This practice may introduce errors as airborne particles are not necessarily deposited homogeneously on the slide and surface-associated fungi collected on tape samples may not be uniformly distributed.

The results of air samples are reported as the total number of spores/ m^3 of air, using the volume of air sampled to convert the data, and recognizable spores are identified and reported as the percent of the total. Surface samples from tape lifts are generally reported as semi-qualitative assessments using a numerical scoring system. For example, < 5 spores / sample scored as a 1+; 5-20 spores /sample scored as a 2+; 20-50 spores / sample scored as a 3+; or: > 50 spores/sample scored as a 4+. Growth and reproductive structures (e.g., hyphae and conidiophores, respectively) are important to note as these structures demonstrate the growth and colonization of fungi on the sampled surface. Additionally, reporting of skin cells, particulate, or other fragments assist in evaluation of the condition of the sampled surface.

3. Additional analytical methods

Analytical chemistry methods (e.g., high performance liquid chromatography [HPLC], gas chromatography with mass spectrophotometry [GC-MS]) can be used to detect ergosterol as indicators of the presence of fungal biomass (Douwes *et al.*, 1999, Pasanen *et al.*, 1999). However, identification of the types of fungi present is not possible and these methods are generally limited to analysis of surface samples. Similarly, detection of (1-3)- β -D-glucan (a structural component of fungal cell walls) as an indicator of fungal biomass can be performed using an enzyme inhibition method (Douwes *et al.*, 1996; Rylander, 1999). Analytical chemistry methods are also used to detect mycotoxins, toxic chemicals produced by fungi. While the presence of these compounds has been associated with spores and reports of adverse human health in exposed building occupants (Sorenson *et al.*, 1987) routine assay for these compounds in samples from indoor environments has not been widely adopted.

Immunochemical methods (e.g., enzyme-linked immunosorbent assay [ELISA]) rely on specific antigen-antibody recognition. This type of assay can be used to detect the presence of specific fungal antigens present in a sample, but currently its use is limited for monitoring of indoor environments (Douwes *et al.*, 1999).

4. Molecular biology

The application of polymerase chain reaction (PCR) amplification for the detection and quantitation of contaminant fungi in indoor environments is developing. This technology relies on the amplification of a known gene sequence that is unique to a particular mold to detect and enumerate an organism of interest in a sample, and it is not dependent on the viability of the collected material. PCR decreases the analysis time and increases specificity and sensitivity, but concerns for inhibition by environmental substances present in samples requires development of additional strategies for sample processing (Cruz *et al.*, 2001a and 2001b). Therefore, PCR is primarily used in research applications (Haugland *et al.*, 2001), but some commercial laboratories offer this analysis for indoor samples.

5. Limitations to current analysis methods

There are limitations to all analysis methods. Culture depends on the ability of every spore to germinate and grow into a recognizable colony. Successful culture depends on the viability of the spore, and on the nutritional and environmental requirements imposed by the laboratory conducting the analysis. No single culture medium or set of environmental conditions will detect all the viable spores in a sample. In addition, fast-growing species will often dominate cultures of environmental samples, preventing the detection of slower growing organisms. These problems may result in underestimation of the fungal population in the sample as only the culturable spores are detected. Because not all mold spores have to be living (e.g., *Alternaria alternata*) to cause allergic effects the detection of total spores using microscopy is useful. However, fungal genera with spores of similar appearance (e.g., *Aspergillus* and *Penicillium*) cannot be distinguished using traditional light microscopy. Therefore, it is recommended that microscopic and cultural methods be used in combination to provide a reasonable representation of fungal populations. Methods

to detect fungal biomass indicators (i.e., ergosterol, (1-3)- β -D-glucan) do not provide information on the populations of fungi and immunoassay methods have relatively poor sensitivity requiring large quantities of antigen to be present before a positive result is obtained. PCR, which has greater sensitivity than other assays and exquisite specificity, currently is hampered by interferences from environmental background, and it only detects the specific organisms for which the assay is designed for.

B. Air sampling

Air sampling is performed to document the concentration and composition of airborne fungi. It is important to remember that molds grow on surfaces and the spores are dispersed from these surfaces as a result of air movement and physical disturbance (e.g., removal of contaminated walls, walking on contaminated flooring). Therefore, negative results obtained from air samples do not ensure that a fungal biocontaminant is not present in the indoor environment. Because there are no recognized instruments for the direct reading of airborne fungal levels in residential or commercial buildings, it is necessary to collect samples in the field for later analysis at the laboratory. A variety of sampling methods have been used in monitoring of indoor environments (Table 11.1).

1. Impactor samplers for culture-based analysis

A variety of air samplers are commercially available (Buttner *et al.*, 2002a; Pasanen, 2001), but the most commonly used for routine indoor air quality monitoring of culturable fungi is a single-stage impactor sampler. The Andersen single-stage impactor sampler (ThermoAndersen Inc., Smyrna, GA) was developed in the late 1950s (Andersen, 1958) and is still widely used, but since the patent on this device has expired other similar samplers are now available. The Andersen sampler requires a vacuum pump, an electrical power source, and a pre-filled agar petri plate. Air is drawn through 400 small holes in a sieve plate that accelerates the air stream. Particles are collected when the velocity of the air through the holes propels the particles with sufficient inertia to leave the air stream and impact onto the agar surface. The distance from the sieve plate to the agar surface is critical, and is controlled by the amount of agar in the petri plate (40ml of agar in a 100 mm petri plate is required for the traditional single-stage sampler). The sampler is usually placed on a tripod at the

Table 11.1
Overview of monitoring methods for mold in indoor environments.

Method	Principle	Analysis	Advantages	Limitations
Air Sampling				
Impaction	deposition onto agar surface	culture	enumeration of culturable airborne fungal spore concentrations; speciation of colonies	dependent on physiological state of organism, selection of growth media and temperature; takes days to weeks for growth and identification; agar surface may become overcrowded with high airborne concentrations
	deposition onto adhesive-coated slide surface	microscopy	enumeration of airborne fungal spore concentrations; not dependent on culture	cannot discriminate between some spores at the genus level and cannot identify to the species level; may become overloaded in conditions of high airborne concentrations
Impingement	deposition into liquid	culture, microscopy, analytical chemistry, molecular biology	enumeration of culturable airborne fungal spore concentrations; variety of analytical methods; can dilute sample to avoid overloading during analysis	difficult to manipulate in the field due to glass construction and liquid sample; may induce increased sampling stress
Filtration	deposition onto filter material	culture, microscopy, analytical chemistry, molecular biology	variety of analytical methods; can dilute sample to avoid overloading during analysis	may result in desiccation of organisms and culturability losses
Surface Sampling				
Contact	direct plating from surface onto agar	culture	inexpensive collection	representative of a limited area due to small contact area; dependent on physiological state of organism, selection of growth media and temperature; takes days to weeks for growth and identification; agar surface may become overcrowded with high
Swab	removal of material from a surface using a sterile swab	culture, microscopy, analytical chemistry, molecular biology	inexpensive collection; can dilute sample to avoid overloading during analysis; variety of analytical methods	representative of a limited area to small sample size
Bulk	removal of building material	culture, microscopy, (analytical chemistry, molecular biology of eluted sample)	variety of analytical methods; can dilute sample to avoid overloading during analysis	destructive method requiring removal of building material from structure; representative of a limited area due to small sample size
Dust	removal of settled dust	culture, microscopy, analytical chemistry, molecular biology	variety of analytical methods; can dilute sample to avoid overloading during analysis	representative of a limited area due to small sample size

breathing zone (i.e., 1.5 meters from the floor) although occasionally the sampler may be placed on a tabletop or a sampling cart during monitoring. The lower limit of detection (LLD) for this impactor sampler operating at 28.3 liters/min. for a 2-minute sampling time is 18 CFU/m³ of air. Increasing the sampling time may reduce the LLD, but the agar surface may become overloaded with increased levels of fungi and the agar surface may dry out leading to increased sampling stress and particle bounce, resulting in underestimation of airborne concentrations.

2. Impactor samplers for microscopic-based analysis

Collection of total airborne fungal spores is generally accomplished using a spore trap sampling device (Buttner *et al.*, 2002a; Pasanen, 2001). The Air-O-Cell is a single-use, portable cassette slit sampling device (Zefon Analytical Accessories, St. Petersburg, FL) that attaches to a vacuum pump. Particles are collected on an adhesive-coated glass cover slip internal to the cassette. The sampling pump is operated at 15 liters/minute for a predetermined interval, generally 5 to 15 minutes). These cassettes can be easily shipped to the laboratory for analysis and there is little concern for cross-contamination between samples, but there is no provision for examining the sample in the field to determine if the slit is overloaded with particles, in which case an additional sample with a smaller volume could be collected. A portable, battery-powered sampler (Burkard Personal Impactor Sampler, Burkard Manufacturing Company Ltd. England, distributed in the United States by Spiral Biotech, Inc., Bethesda, MD) collects short-term (1 to 15 minute) samples through a slit onto an adhesive-coated glass slide and operates at 10 liters/min. This sampler is small and lightweight with an internal rechargeable battery, and the glass slide can be examined to determine the amount of sample collected thereby avoiding overloading of the viewing area.

3. Filter sampling

Collection of airborne particles using filter cassettes provides a means to sample for extended periods of time without overloading an agar surface or microscopic field of view (Buttner *et al.*, 2002a; Pasanen, 2001), and filter sampling has been used to detect airborne mycotoxin (Pasanen *et al.*, 1993). However, some concern for dessication and subsequent loss of viability is inherent in the method when used with culture-based analy-

sis. The collected material is returned to the laboratory for processing in a manner similar to that for bulk or dust samples (see below).

4. Gravity / settle plate sampling

Gravity or settle plate sampling is the exposure of agar-filled petri plates or greased slides to the air. There is no volumetric measurement of the air from which spores are deposited. Therefore, this is a non-quantitative sampling method. This type of sampling has been used for assessing airborne fungal presence because it does not require expensive sampling equipment and can be performed by building occupants. However, it relies on the settling of airborne particles and ignores the differential settling of spores due to differences in size and shape. In addition, air current fluctuations over the sampling surface can drastically change collection characteristics. Studies have shown that gravity/settle plate sampling poorly represents the populations of microorganisms in aerosols (Sayer *et al.*, 1969, and 1972; Solomon, 1975) and it is not recommended for use to determine fungal populations in indoor environments.

5. Limitations to air sampling

Unfortunately, all currently available methods of air sampling often underestimate the presence of fungal contaminants in indoor environments (ACGIH, 1999) due to issues with biological and physical efficiency of the instruments. Biologically, a culture-based collection method may stress the organism(s) resulting in the inability of the colony to grow under the conditions provided. Physically, the sampling method may not efficiently collect the particle size of the contaminate organism or permit discrimination from other organisms or abiotic material. Therefore, investigations of indoor environments should combine the use of a culture-based air sample and a non-viable, spore trap method, and the investigation should include surface sampling for the determination of possible sources of fungal contamination.

C. Source/surface sampling

Source/surface sampling can be used to identify the kinds of mold colonizing surfaces and can provide an indication of the reservoir of fungal material that may be dispersed into the air. A variety of source/surface sampling methods are available to detect mold indoors (Table 11.1).

1. Culture-based surface sampling

One traditional method for evaluating surfaces for microbial contamination is the use of a contact plate in which a convex agar surface is pressed against the surface to be sampled. After incubation, colonies that grow on the agar are counted and identified. Commercially prepared contact plates are available which can contain any one of a variety of culture media or the laboratory conducting the analysis may prepare the plates for the specific monitoring project. Results are often extrapolated to larger surfaces providing semi-quantitation of contamination. However, such this information is only valid if there is uniform distribution of organisms, which rarely occurs for fungal contamination indoors and this practice can over or under estimate the amount of contamination present.

Irregular, uneven, textured, and smooth surfaces are often sampled using commercially available, sterile swabs. Swabs may be used directly or pre-moistened in sterile liquid. The swab is wiped across the surface to be sampled and then placed into a sterile transport container and shipped to the laboratory for analysis using culture. Semi-quantitative results can be achieved but, as with contact plates, extrapolation to surfaces beyond those actually sampled is generally not possible due to uneven distribution of fungi on surfaces.

Bulk sampling refers to the collection of sections of building materials and furnishings (e.g., ceiling tile, wallpaper, wallboard, carpet) that are transported in labeled plastic bags to the analytical laboratory where the material can be analyzed by microscopy or by culture. For culture analysis, pieces of the sample are suspended in buffer, and mixed to dislodge fungal material. The buffer can then be used to inoculate a variety of growth media. Microscopic analysis is generally performed using a stereomicroscope to scan the surface of the material followed by the collection of transparent tape samples from selected areas of the bulk sample. Removal of plug segments of insulation materials (Buttner *et al.*, 2002b) or flooring (Buttner *et al.*, 1999) has been used to quantify fungal contamination in air handling duct materials, but bulk sampling is a destructive method necessitating the removal of material from the site where the sample is collected.

A non-destructive method that is useful in identification and quantitation of surface-associated fungi is dust sampling. Dust sampling involves collecting material from porous surfaces (e.g., carpet, air han-

dling duct liner) and non-porous surfaces (e.g., vinyl flooring, horizontal surfaces of bookcases, cabinets). Samples are collected with a commercial single-use filter cassette using a vacuum device or an adapter to a traditional vacuum cleaner. After collection, the samples are usually sieved to remove debris (e.g., hair) and the fine dust is weighed and suspended in a detergent solution (e.g. sterile water with 0.05 percent tween) with serial dilution and plating to culture media (Macher, 2001). Although the dust is usually analyzed by culture, some laboratories will also examine the sample microscopically.

2. Microscopy-based surface sampling

Transparent adhesive tape can be used to sample visible fungal growth on surfaces. The tape is pressed against the visible growth and then mounted on a glass slide for microscopic examination. The method is qualitative providing a means to observe recognizable fungal structures sufficient for identification of many fungal genera without the need for culture. This method has been successfully used for identifying fungal contamination on carpet backing and woven baskets when the fungi were not readily culturable (Kozak *et al.*, 1980).

D. Interpretation of Data

No exposure limits have been established for airborne or surface-associated fungi in commercial or residential indoor environments. Several years ago, some guidelines for interpretation of data were published with the general consensus that indoor fungal levels should be lower than those outdoors and the populations should be similar indoors and outdoors. In a review of published reports, Rao *et al.*(1996) noted that many investigators agree with Miller et al (1988) that the presence of some fungi (e.g., pathogens and certain toxigenic fungi) are unacceptable in indoor air. However, the American Conference of Governmental Industrial Hygienists does not support any numerical criteria for interpreting air or surface data, but discusses interpretation of data that are indicative of environmental contamination and exposure of building occupants (Burge *et al.*, 1999). This document also presents perspectives on comparisons of data with known data bases, comparisons of complaint and non-complaint areas within buildings, and on indoor/outdoor ratios. The lack of adequate dose/response data contributes to the current disagreement among those

attempting to formulate guidelines for acceptable environmental levels of fungi. However, as sampling and analytical protocols are developed to allow estimation of actual exposure and standardized protocols are established, it is likely that reliable assessments of the risk associated with fungal exposure will be obtained.

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