

Rosengarten, Smith & Associates presents

Guidelines for a Professional Moisture Investigation

PART 2- SAMPLING

Introduction

Mold reproduces by producing microscopic spores. When mold spores land on most wet building materials, they may begin growing and digesting the material on which they are growing.¹ Spores may remain viable for years. Allergens found naturally in and on spores may remain allergenic for years.² Some mold species can produce several toxins while others produce mycotoxins only under certain environmental conditions. The presence of mold in a building does not necessarily mean that mycotoxins are present.³

All species of mold have the potential to adversely affect health. Mold can produce allergens that can trigger allergic reactions or even asthma attacks in people allergic to mold.⁴ According to the Texas State Comptroller's Office, about 10% of the general population is severely allergic. For them, exposure to household mold, if prolonged and intense enough, can cause asthma, coughing, headaches, eye irritation, dizziness and even death.⁵ Specific individuals appear to be at a higher risk for adverse health effects of mold: infants and children, the elderly, patients with compromised immune systems (such as people with HIV, infections, liver disease, or undergoing chemotherapy), pregnant women, and individuals with existing respiratory conditions such as allergies, multiple chemical sensitivity and asthma.⁶ The American Academy of Pediatrics Committee on Environmental Health reports that infants under one year of age should not be exposed to

chronically moldy, water-damaged environments.⁷

If mold testing is performed, it is critical that the investigation goals are clearly understood, logical and achievable. Testing for mold should not be conducted unless an objective case can be made for it as a necessary part of solving the problem. Experienced and competent investigators should be able to justify any recommended mold sampling with a clear statement of how the test results will be used in determining solutions to the problem.⁸

Presently, no standards exist to determine a safe, a healthy or a clean level of surficial or airborne mold. Mold testing procedures were not developed to determine whether an occupied space is safe. Indoor mold testing procedures were developed to identify locations where mold is growing or where it has grown - mold reservoir locations or mold amplification areas. No one has published typical conditions for homes, offices and schools. There is no published baseline for comparison. Research is ongoing to establish health-based levels of mold, but it might be many years before they are established.⁹

Choosing a Laboratory

Microscopic identification of mold requires considerable expertise. The American Industrial Hygiene Association (AIHA) offers accreditation to microbial laboratories under the Environmental Microbiology Laboratory Accreditation Program (EMLAP). Accredited laboratories must participate in quarterly proficiency testing known as the Environmental Microbiology Proficiency Analytical Testing (EMPAT) Program.¹⁰

Surface Sampling for Mold

Tape Lift Technique

Cellophane tape sampling is performed by applying the adhesive side of clear packaging tape on a surface to lift off visible mold. The mold spores on the tape are characterized and counted by microscopic analysis. The advantage of tape sampling is that microscopic analysis can be conducted quickly, as opposed to culturing fungi, which may take many days. This technique may be of significant benefit in determining if visibly affected surfaces have mycotoxin-producing species of mold.

Bulk Sampling

Bulk sampling consists of collecting a piece of an affected building material. The material can be examined directly by microscope or cultured and analyzed. A general guideline for cultured bulk samples is that less than 5,000 CFU/g (colony forming units per gram of material) are normal and 5,000 -10,000 CFU/g are elevated. Elevated levels indicate that further investigation should be conducted. Microbial levels over 10,000 CFU/g have the potential to significantly contribute to airborne populations.¹¹

Swab Sampling

Swab sampling consists of wiping the surface of a fabric, building material or HVAC system component. The mold on the swab may be characterized and quantified by microscopic analysis or cultured for viable mold. A general guideline for cultured swab samples is that less than 200 CFU/cm² (colony forming units per square centimeter) is normal. Microbial levels between 200-500 CFU/cm² are elevated. Elevated levels indicate that further

investigation should be conducted. Microbial levels over 500 CFU/cm² (3,225 CFU/per square inch) are considered to have the potential to significantly contribute to airborne populations and require remedial action.¹²

Air Sampling

Determining When Air Sampling is Warranted

Interviews with building occupants complaining of adverse health effects may identify suspect areas in a building. This information alone may provide what is needed to locate water-damaged areas.

Suspect areas should be visually inspected, including walls behind furniture and roofing materials above ceiling tiles. HVAC units should be checked for moisture in condensate pans, plenums and ductwork. If the visual investigation indicates no visible mold but the building space smells moldy, mold may be hidden behind wallpaper or in areas such as pipe chases and underground passageways that contain electrical and plumbing utilities (utility tunnels).

Air monitoring may be necessary in certain situations, including: 1) if an individual has been diagnosed with a fungal exposure, 2) if it is suspected that ventilation systems are affected, or 3) if the presence of mold is suspected but cannot be identified by a visual inspection or bulk sampling.¹³

Air sampling should not be part of a routine assessment.¹⁴ Numerous states recommend that air sampling not be performed during an initial assessment.¹⁵

Air Sampling Plan

Air sampling provides information for the moment in which the sampling occurs, much like a snapshot.¹⁶ It is important to get as much information as possible from the building occupants, building maintenance personnel and building owner or management company to develop a sampling plan appropriate to the problems experienced.

Budgetary considerations may limit the number of samples to be taken. Airborne mold sampling should not be performed if the number of samples is restricted so that testing will not provide the information needed to achieve the goal of the sampling plan. An insufficient number of samples will not confirm any hypothesis. A sampling plan must be based on a practical methodology that is directed toward solving the problem at hand.

Two air sampling methods are commonly used to collect airborne particles: impactor cassettes and vacuum/culture techniques. Impactor cassettes collect particles from air drawn by a pump. Air is accelerated through a venturi opening and impacted onto a sterilized, oil-coated slide in a cassette casing. Microscopic analysis is conducted on the slide, providing quick identification and quantification of airborne particulate materials, including viable and non-viable mold spores. These cassettes also collect pollen, insect parts, skin flakes and other airborne particles. Air-O-Cell[®] and Cyclex-d[®] are popular brand name cassettes.

The vacuum/culture technique utilizes a sieve-type sampler for the collection of viable mold spores. A sieve-type air sampling device commonly used is the Andersen¹⁷ sampler, which uses a vacuum pump to draw air through a radial pattern of 300 small pores, impacting particles onto the surface of microbial growth medium or agar.¹⁷

Media used for culturing fungi are numerous. They include 2% malt extract agar, Sabouraud dextrose agar, dichloran 18% glycerol agar, rose bengal agar, Littman Oxgall agar and many others. At least two apparently irreconcilable dichotomies must be addressed by the person trying to select a single medium for indoor fungal study: that no one medium will optimize growth of both hydrophilic (water-loving) mold (e.g. *Stachybotrys*) and hydrophobic (preferring relatively dry conditions) mold (e.g. *Eurotium*, *Wallemia*) and that the best media for identifying airborne organisms are also the most problematical for colony overgrowth and formation of spurious satellite colonies in shipping and handling.¹⁸ The best alternative may be to consult with the laboratory and ask for their recommendation as to the best medium based on the sampling to be performed.

Because of the variety of mold species and the limitations of the different sampling media, using both impactor cassettes and vacuum/culture technique with a general use agar can provide a comprehensive approach to determine an indoor amplification of mold. While hydrophilic genera like *Stachybotrys* will not be identified by a general use agar, its spores on an impactor cassette slide will be readily visible under a microscope based on its size. Alternative forensic lab analysis techniques, such as polymerization chain reaction (PCR)

testing, are rapidly emerging and may soon provide comprehensive methods for identifying mold.

An outdoor sample is collected to compare mold outdoor levels to indoor levels. The outdoor sampling should take place at least 6 meters (20 feet), and preferably 10 meters (33.3 feet), upwind of the building.¹⁹ At least two samples should be taken at entrances to the building or at fresh air intakes (in commercial buildings).²⁰

Factors Affecting Air Sampling Results

Sampling can be affected by numerous factors. If an outdoor count is taken during rainfall, the spore count may be suppressed. After a rain event flora will sporulate and the spore count may rise significantly. Changes in temperature and humidity affect airborne fungal counts. Air sampling provides information only for the moment in time in which the sampling occurred.

Movement indoors can affect spore counts. Merely walking on carpets or dust-laden hardwood, tile and vinyl flooring can increase spore counts. Dogs and cats may track in grass clippings, decayed leaves and soil that have the potential to affect analytical testing. The HVAC system can affect spore counts. It is not uncommon to have airborne sampling analytical results that are significantly different when the HVAC system is not operating and then when it is.

Air sampling methods for some fungi are prone to false negative results and, therefore, cannot be used to definitely rule out contamination.²¹ *Aspergillus* and *Penicillium* mold spores identified in cassettes are difficult to distinguish by direct microscopic analysis, leading to non-specific results. Culture media may be overgrown with microorganisms other than mold, such as bacteria.

Analyzing Air Sampling Results

Air samples should be evaluated by means of comparison (indoors to outdoors) of airborne concentrations and by fungal type (genera and species). In general, the types and relative compositions of fungi should be similar indoors (in non-problem buildings) to the outdoor air. Differences in the relative compositions or types of fungi found in air samples may indicate that moisture sources and resultant fungal growth may be problematic.²² Similarly, a species found indoors in a proportion of total spora much greater than its outdoor proportion is usually proliferating indoors.²³

No scientifically peer-reviewed research is readily available to definitively provide typical and/or acceptable levels of culturable or non-culturable indoor fungal bioaerosols. Various guidelines for interpreting mold-sampling data have been developed, but scientific consensus suggests that it is overly simplistic and inappropriate to rely solely on a comparison of test results to any numerical criteria. The Minnesota Department of Health regards numerical guidelines for mold as arbitrary and does not support their use as the sole basis of determining if an environment needs to be corrected.²⁴

A database prepared by Mycotech Biological, Inc. (MBI) generally suggests that total bioaerosols within indoor environments are typically below 2,000 particles per cubic meter. This level is not intended to represent a threshold value having a medical significance, nor is it necessarily representative of an acceptable limit for a living environment. Rather, it is intended to be a threshold to suggest further investigation for potential conditions that could lead to indoor fungal amplification. To date, there are no data that support a threshold limit or dose-response relationship for exposure to fungal aeroallergens.

A recommended guideline limit for culturable fungal bioaerosols is 300 CFU/m³ (colony forming unit per cubic meter) total and 50 CFU/m³ for individual genera, except *Cladosporium* or other tree or leaf fungi, which may be acceptable up to 500 CFU/m³ in summer.²⁵ Identification of *Aspergillus*, *Penicillium* and genera of other numerically significant fungi is recommended.²⁶ The persistent presence of significant numbers of toxigenic fungi indicates that further investigation is warranted.

Endnotes

1. *Mold Remediation in Schools and Commercial Buildings*, U.S. Environmental Protection Agency, Office of Air and Radiation, Indoor Environments Division, EPA 402-K-01-001, March 2001, page 2.
2. Ibid., page 43.
3. Ibid., page 42.
4. Ibid., page 2.
5. Fiscal Notes, *Home Sweet Home-Without Insurance?* State Comptroller's Office, State of Texas, March 2003, page 6.
6. *Mold in My Home: What Do I Do?* Indoor Air Quality Fact Sheet, California Department of Health Services, March 1998, pages 2-3.
7. *Toxic Effects of Indoor Molds (RE9736)*, policy statement by the American Academy of Pediatrics, Volume 101, Number 4, April 1998, pages 712-714.
8. *Recommended Best Practices for Mold Investigations in Minnesota Schools*, Minnesota Department of Health, Environmental Health Division, Indoor Air Unit, November 2001, page 8.
9. *Mold Remediation Guidelines for Texas*, Texas Association of Builders Building Standards Task Force, 2002, page 5.
10. *Guidelines on Assessment and Remediation of Fungi in Indoor Environments*, New York City Department of Health, Bureau of Environmental and Occupational Disease Epidemiology, 2000, page 9.
11. *Investigating and Mitigating Microbiological Contamination in Buildings*, presentation by the MidAtlantic Environmental Hygiene Resource Center at the University City Science Center with support from the United States Environmental Protection Agency and the United States Public Health Service, Philadelphia, PA, May 4-5, 1995, page 30.
12. Ibid.
13. *Healthy Homes Issues: Mold*, U.S. Department of Housing & Urban Development, Healthy Homes Initiative Background Information, External Draft Review, Version 2, October 2, 2001,

page 7, references the 2000 *Guidelines on Assessment and Remediation of Fungi in Indoor Environments*, New York City Department of Health, Bureau of Environmental and Occupational Disease Epidemiology.

14. *Guidelines on Assessment and Remediation of Fungi in Indoor Environments*, New York City Department of Health, Bureau of Environmental and Occupational Disease Epidemiology, 2000, page 8.
15. *Mold in My Home: What Do I Do?*, Indoor Air Quality Information Sheet, Arizona Department of Health Services, page 2; *Mold in My Home: What Do I Do?*, Indoor Air Quality Information Sheet, California Department of Health Services, March 1998, page 3; *Mold In the Home: Health Concerns*, Connecticut Department of Public Health, Division of Environmental Epidemiology & Occupational Health, page 3; *Mold In the Home*, Mississippi State Department of Health fact sheet, page 3; *Mold in My Home: What Do I Do?*, Indoor Air Quality Information Sheet, North Dakota Department of Health, June 1999, page 4.
16. *Mold Remediation in Schools and Commercial Buildings*, U.S. Environmental Protection Agency, Office of Air and Radiation, Indoor Environments Division, EPA 402-K-01-001, March 2001, page 25.
17. *Fungal Contamination in Public Buildings: A Guide to Recognition and Management*, Health Canada, Federal-Provincial Committee on Environmental and Occupational Health, June 1995, Glossary, page 38.
18. *Ibid.*, pages 72-73.
19. *Ibid.*, page 16.
20. *Singapore Guidelines for Good Indoor Air Quality in Office Premises*, Institute of Environmental Epidemiology, Ministry of the Environment, October 1996, page 42.
21. *Guidelines on Assessment and Remediation of Fungi in Indoor Environments*, New York City Department of Health, Bureau of Environmental and Occupational Disease Epidemiology, 2000, page 8.
22. *Ibid.*, page 9.
23. *Fungal Contamination in Public Buildings: A Guide to Recognition and Management*, Health

Canada, Federal-Provincial Committee on Environmental and Occupational Health, June 1995, page 17.

24. *Recommended Best Practices for Mold Investigations in Minnesota Schools*, Minnesota Department of Health, Environmental Health Division, Indoor Air Unit, November 2001, page 20.

25. *Fungal Contamination in Public Buildings: A Guide to Recognition and Management*, Health Canada, Federal-Provincial Committee on Environmental and Occupational Health, June 1995, page 7.

26. *Ibid.*, page 19.