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Sampling for indoor fungi

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Background: A great deal of concern has arisen recently regarding the potential adverse effects of indoor fungi. Our understanding of this complex problem has been hampered by a lack of standardized protocols for performing an indoor assessment for fungi. Without such standards, it is difficult to compare results from one study with those from another or to measure the effect of indoor fungal contamination on a building and its occupants.

Methods: We reviewed the medical literature and here describe a hypothesis-driven approach to planning, sampling, and interpreting the results of indoor assessments for fungi.

Results: Fungi cause 3 primary adverse effects: (1) they can damage a building, (2) they can render a building unpleasant to live in by looking and smelling bad, and (3) they might cause adverse health effects in sensitive individuals. Sampling methods used to test hypotheses include air sampling for spores, measurement of allergens in house dust, and determination of microbially generated volatile organic compounds, ergosterols, glucans, and mycotoxins, as well as environmental conditions that lead to fungal contamination.

Conclusions: Standardized approaches for performing and reporting assessments of indoor fungi are essential if our understanding of this complex field is to improve. (*J Allergy Clin Immunol* 2004;113:189-98.)

Key words: *Fungi, mold, Stachybotrys species, environmental sampling, surface sampling, air sampling, ergosterol, glucan, mycotoxin, microbially generated volatile organic compounds*

A search of the World Wide Web for the terms "toxic mold," "black mold," or "Stachybotrys" produces links to numerous sites. Companies that test for fungi or that provide remediation account for the largest number of links, confirming that this is a growing industry.¹ The effect of

Abbreviations used

CFU: Colony-forming unit

COC: Chain of custody

EIA: Enzyme immunoassay

MVOC: Microbially generated volatile organic compound

indoor mold on health is highly controversial. Within the medical and academic communities, controversies exist about the most basic questions²⁻⁵: (1) whether exposure to indoor fungi causes health effects, (2) the identity of the best method to measure exposure, and (3) what should be done if fungal exposure is present. For assessment and remediation companies, the "health effects" question appears to be settled. The US Environmental Protection Agency takes a pragmatic view, sidestepping the controversy by recommending remediation of all fungus-contaminated buildings.^{6,7}

The public debate over indoor fungi occurs in a context in which there are no standard protocols for measuring them or interpreting measurements when done. This makes it virtually impossible to determine the relevance of any reported fungal exposure with a particular person's symptoms. Our goal here is not to sort out the controversies about the health effects of fungi but rather to suggest guidelines for their measurement. Such standardized protocols are a necessary prerequisite for establishing a foundation that can lead to organized scientific investigation of the issue.

WHY SAMPLE FOR FUNGI?

In 1859, Louis Pasteur disposed of the theory of spontaneous generation by showing that airborne microscopic organisms account for biologic growth on previously sterile media.⁸ The ability of a microorganism to travel through air requires durability and special aerodynamic adaptations. Many microorganisms prepare for flight by encapsulating themselves in tough sheaths, thus becoming spores. Blackley first associated exposure to outdoor fungal spores with rhinitis.⁹ More recently, studies of indoor air have established that exposure to indoor aeroallergens also is associated with symptoms. Initially, dust mite and cat allergens^{10,11} and more recently cockroach and fungal allergens have been related to symptoms in sensitive individuals.¹²⁻¹⁴ This increased interest

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in indoor allergens has fueled a parallel interest in developing standardized methods for environmental sampling.¹⁵ Consequently, a number of books that describe fungal sampling in great detail have helped us to develop the following protocols.^{16,17}

There are 3 reasons why there should be concern about fungi in the indoor environment: (1) potential health effects of exposure to fungi and their metabolic products,¹⁸ (2) the effect of fungal contamination on the structural integrity of a building,⁶ and (3) the negative aesthetic effects fungi can produce both visually and on the human olfactory systems.¹⁹⁻²¹ Although the issue of whether exposure to indoor fungi causes adverse health effects is controversial,¹⁴ there is no question that a seriously contaminated building can suffer structural damage and that a foul-smelling, fungus-filled building is aesthetically displeasing.⁶ Controversies about health effects aside, the latter 2 reasons are sufficient to merit concern when an environment is found to have fungal contamination.

Persons who have concerns about structural damage or the aesthetic effects of indoor fungi should seek advice from an environmental consultant. The advice of a health care professional is needed if health effects are a concern. Because adverse effects of exposure to fungi are frequently mentioned in the media, symptomatic individuals might conclude (rightly or wrongly) that fungi are the cause of their symptoms. Physicians who see such individuals should consider their concerns as seriously as any other health problem.

THE SAMPLING PROCESS

Hypothesis development and testing

Hypotheses concerning the likely role of fungal exposure on adverse effects should be developed before any sampling.²² If building damage caused by fungal contamination is suspected, a reasonable hypothesis would be that fungi are present and that they are harming the building. Aesthetic concerns might lead to the hypothesis that stains or undesirable smells are caused by the presence of fungi. Concerns about health effects are more complicated because they involve a chain of hypotheses: (1) fungi are present in the environment, (2) fungal spores or metabolites travel from source locations to affected persons, (3) sufficient exposure is present to cause symptoms, (4) affected individuals are sensitive to the exposure, and (5) symptoms are caused by the exposure. This series of hypotheses all need to be tested before one can unequivocally conclude that fungi are the cause of symptoms.¹⁴

The 3 categories of environmental assessment include the following: (1) identification of factors leading to fungal growth, (2) identification of paths from sources of fungal growth to affected persons, and (3) occupant exposure to fungi and their metabolites. Determination of patient sensitivity to fungi and assessment of the relationship between that sensitivity and symptoms should be determined by health care professionals guided by the results of the environmental sampling.

It is helpful to ask the occupants where they believe the problem is located to narrow the types of sampling to be performed.¹⁴ A checklist that can be used to elicit this type of information is in the supplemental materials related to this article on the Journal's Web site (Table E1 in Online Repository at www.mosby.com/jaci). It also is helpful to get the results of previous sampling, and it might help to consult with other current or former occupants and maintenance personnel who might know about previous problems with the building. Differences in susceptibility to environmental exposures might lead to discrepant symptom histories. The hypotheses and sampling strategy should address the concerns and beliefs of all building occupants. In many cases it is sufficient simply to determine whether fungal contamination of a building is causing damage or an unpleasant smell. Establishment of a clear link between symptoms and a contaminant might be difficult, and depending on the hypotheses, it might be unnecessary.²³ The more the environmental consultant knows at the outset, the less additional sampling will be needed to test the hypotheses.

General principles of sampling

Sampling should be performed by using validated methods and should be planned so that the minimum amount of sampling and interpretation is done to meet the information needs.¹⁶ The person doing sampling should know how to calibrate and properly use his or her instruments.²⁴ Fortunately, most of the instruments and methods described here use standard protocols that are described in the various devices' instruction manuals.^{16,17,22,25}

The main reason for sample collection when fungal contamination is suspected is to detect, quantify, and identify any fungi that might be present. Because airborne biota might include microorganisms (both fungi and bacteria), pollen, fragments from animals, and manmade particulates (eg, diesel particles and soot), selection of appropriate collection techniques depends on the hypothesis to be tested. There are 2 primary collection techniques for bioparticulates: air and surface sampling. Sources of samples might include viable or nonviable spores from air, surfaces, or bulk materials. Antigens or toxins might be measured from vacuum samples by using enzyme immunoassay (EIA) of allergen-antigen content. Table I lists air-sampling devices, and Table II is a list of air-collection devices with their suppliers and some laboratories that perform analyses. A typical sampling protocol also should include measurement of temperature and relative humidity in all major areas of the building.

Several general sampling principles include the following:

- Air samples always should include an outdoor sample as a reference.
- Samples should be collected both before and after potential sources of contamination are disturbed.
- Investigators should account for the effect samplers and inspection personnel might have on the samples being taken.

TABLE I. Types of air samplers and features

Type of sampler	Advantages	Limitations
Viable spore samplers		
Slit impact onto agar	Determination of viable organisms Species determination possible Continuous operation over time	High flow rates might desiccate agar Aggressive genera overwhelm slower-growing genera Counting is difficult in highly contaminated situations Clumps of organisms appear as single colonies Results must wait for colonies to grow
Hole impact onto agar	Determination of viable organisms Species determination possible	Multiple samples necessary for adequate lower limit of detection High flow rates might desiccate agar Aggressive genera overwhelm slower-growing genera Counting is difficult in highly contaminated situations Clumps of organisms appear as single colonies Results must wait for colonies to grow
Multistage hole impact onto agar	Separation of organisms by size Determination of viable organisms Species determination possible	Very labor intensive when performing multiple samples Multiple samples necessary for adequate lower limit of detection High flow rates might desiccate agar Aggressive genera overwhelm slower-growing genera Counting is difficult in highly contaminated situations Clumps of organisms appear as single colonies Results must wait for colonies to grow
Nonviable spore samplers		
Continuous slit impact onto coated slide	Spore enumeration Larger sample volume Ease of counting Nearly immediate result Lower limit of detection Continuous sampling	Species determination not possible No determination of viable organisms Time determination is semiquantitative
Discontinuous slit impact onto coated slide	Spore enumeration Larger sample volume Ease of counting Nearly immediate result Lower limit of detection Exact time possible	Species determination not possible No determination of viable organisms Discontinuous collection might miss some events
Single-use cassette impact onto coated surface	Spore enumeration Larger sample volume Ease of counting Nearly immediate result Lower limit of detection Exact time possible	Species determination not possible No determination of viable organisms Discontinuous collection might miss some events Additional expense associated with disposable materials

TABLE II. Manufacturers or sources of indoor fungal sampling equipment

Common name	Type of sampler	Collecting media	Manufacturer or supplier*	Comment
Allergenco MK-3	Nonviable slit impactor	Grease-coated slide	2	Portable Hirst spore trap
Anderson single stage	Viable multihole impactor	Agar on petri dish	3	No size differentiation
Anderson 2 stage	Viable multihole impactor	Agar on petri dish	3	Separates particles by size into 2 fractions
Anderson 6 stage	Viable multihole impactor	Agar on petri dish	3	Separates particles by size into 6 fractions
All Glass Impinger	Viable multihole impactor	Liquid	1, 7, 15	
Mattson-Garvin Air Sampler	Viable slit impactor	Agar on petri dish	11	
Burkard Viable Sampler	Viable multihole impactor	Agar on petri dish	5, 6, 16	Portable single-stage viable collector
Burkard 24 Hour Indoor Sampler	Nonviable slit impactor	Grease-coated slide or tape	5, 6, 16	Portable Hirst spore trap
Dry cyclone sampler	Cyclone collector	2-mL Eppendorf tube	5, 16, 15	Very low efficiency for particles less than 10 μ m
Spin Con	Tangential impactor	Liquid media	14	Sampling rates greater than 1000 L/min
Wetted Cyclone Sampler	Tangential impactor	Liquid media	5, 14	
Personal Sampler	Filter	Filter cassette	8, 9, 12, 13, 15, 18	
Lanzoni Indoor Collector	Nonviable slit impactor	Grease-coated slide or tape	10	Portable Hirst spore trap
Rotorod	Impaction onto moving rod	Grease-coated Lucite rod	17	
Air-O-Cel	Impaction onto coated surface	Cellulose acetate-coated glass	8, 15, 18	Single-use sampler cassette
SAS	Viable multihole impactor	Agar on petri dish	4	Portable single-stage viable collector

*Manufacturers or suppliers: 1, Ace Glass Inc, www.aceglass.com; 2, Allergenco, CIH Equipment Company, Inc, <http://www.cihequip.com/default.htm>; 3, Anderson Sampler; Thermo Electron Corp, <http://www.anderseninstruments.com>; 4, Bioscience Int, <http://www.biosci-intl.com/>; 5, Biotest Diagnostics Corp, www.biotestusa.com; 6, Burkard Manufacturing Co Ltd, <http://www.burkard.co.uk>; 7, Corning Inc, www.scienceproducts.corning.com; 8, Environmental Monitoring System, www.emssales.com; 9, Gelman, Pall Corporation, <http://www.pall.com/laboratory.asp>; 10, Lanzoni. SRL; www.lanzoni.it; 11, Mattson Garvin Air Samplers, <http://www.mattson-garvin.com/front.htm>; 12, Millipore Corp, www.millipore.com; 13, Sartorius North America, www.sartorius.com; 14, Sceptor Industries Incorporated, www.sceptorindustries.com; 15, SKC Inc, www.skcincl.com; 16, Spiral Biotech Inc, www.spiralbiotech.com; 17, Surveillance Data Inc, www.sdi.com or www.pollen.com; 18, Zefon Analytical Associates, www.zefon.com.

Chain of custody

Chain of custody (COC) documentation of a sample from collection through final analysis is essential. This ensures that the results correspond to the described sample. Breaches in the COC might lead to questions concerning result validity. This is particularly important in legal cases, in which results might be alleged to be fraudulent. Documentation of the COC should be done as carefully as the sample collection itself. The following steps should be used to ensure that an unbroken chain is established from source to analysis:

- The person collecting the sample should record his or her name and the time, location, and collection method used.
- The sample should be given an identification number and sealed in an appropriate container for transportation to the analytic laboratory, and the COC form should be signed.
- Transfer of samples from one person to another should be carefully documented. In this way an unbroken chain can be established from source to analysis. (Refer to the supplementary materials for a sample of our COC form.)

SPECIFIC SAMPLING METHODS

Identification of contamination sources

Visual site reconnaissance. The purpose of a visual walk-through is to identify sources of fungal contamination. This is most efficiently done by focusing on locations of concern raised by the occupants, as well as common problem areas near moisture sources. It helps to plan the walk-through before going to a building. A diagram of the building can expedite this planning. For large buildings, such as schools, it might be helpful to obtain a blueprint along with engineering schematics so that electrical, water, and air-handling systems can be located. Less formal hand-drawn diagrams guided by the occupant generally are sufficient for residential buildings. Each room should be described functionally along with problem areas reported by occupants. This type of "site reconnaissance" improves the efficiency of the initial visit.

Identification of moisture sources. Identification and remediation of moisture sources could lead directly to long-term solutions of mold problems.⁶ Moisture sources can be divided into 2 types: condensation and intrusion. Condensation occurs when air comes into contact with a surface that has a temperature of less than the dew point

of the air, which is defined as the temperature at which air has 100% humidity. Dew point can be determined by measuring air temperature and relative humidity and by using conversion charts.²⁶ For porous surfaces, temperature readings can be used to identify potential condensation inside a wall cavity.

Intrusion occurs when water enters a building from external sources, such as leaks, floods, or groundwater penetration. Moisture content resulting from water intrusion can be measured with specifically designed instruments (Delmhorst Instrument Co, Hauppauge, NY, and Tramex Ltd, Dublin, Ireland). Measurement of internal wall and surface moisture can be used to locate and track both external and internal water intrusion and areas of chronic saturation that are conducive to fungal growth.

Surface sampling. Surface sampling can determine whether a stain has resulted from fungal growth or some other problem. For example, discolorations associated with leaks might be due to dissolved materials in the water accumulating on the surface as the water evaporates. Black material that accumulates near air vents might be caused by soot. An environmental consultant who is familiar with the appearance of these types of discolorations should be able to discern areas of fungal contamination on a surface, minimizing the number of surface samples needed.

The most common surface sampling technique is tape-lift sampling. First, a 2-in piece of clear (not frosted) cellophane tape is carefully placed onto the suspect area. The tape is then stained and placed sticky side down on a microscope slide. The presence of fungi can be confirmed, genera can be identified, and a semiquantitative estimation of the amount of each taxa can be determined. Many commercial devices for taking tape samples are available (QuanTEM Laboratories LLC, Oklahoma City, Okla; Bio-Tape, SKC Inc, Eighty-Four, Pa; MOLDetect Laboratory, Lenexa, Kan).

Viable spore sampling of a discolored area is more time consuming than tape-lift sampling because it depends on fungal growth. The typical sampling method is to use a device that combines a sample collector, such as a cotton swab or plastic loop, with a stabilization media for transport. The advantage of a fungal culture is that it can be used to identify species, whereas the tape-lift method is accurate only to the genus level. The disadvantages are that it takes longer, rapidly growing species tend to dominate, and some genera, such as *Stachybotrys* species, grow poorly on most media.²⁷

Surface dust can be tested for fungi by using either the tape-lift method or vacuum collection for culture and estimation of colony-forming units (CFUs). It is reasonable to use the tape lift initially to confirm the presence of spores followed by culture identification if more specific information is needed.

Surface data might be reported as CFUs per square centimeter or semiquantitatively on an arbitrary scale. Any scale that is used should be clearly defined in the report. Interpretation depends on extrapolation of information from a limited sample. The total fungal load on a

surface depends on the concentration and area over which it extends. For clarity and context, it is good practice for the investigator to take detailed notes, and ideally, a photograph of the sampled surface is useful to document its location and size.

METHODS TO IDENTIFY A PATH FROM SOURCES TO OCCUPANTS

If the hypotheses being tested include health effects, it is necessary to identify paths through which airborne fungi or their products travel from sources to locations where occupants experience symptoms.

Air movement

Ventilation occurs when fresh air entering a building replaces indoor air, thus removing bioaerosols. It is measured in terms of the number of air exchanges per hour. If the production of an aerosol exceeds its removal rate, its concentration will increase until equilibrium is reached. Failure to introduce sufficient fresh air might lead to increased respiratory symptoms for building occupants. Measurement of ventilation can determine whether the amount of introduced fresh air is adequate for the number of building occupants (Pro-Am Safety, Inc, Warrendale, Pa; Anemometers, AFC International, Inc, DeMotte, Ind; Technika, Phoenix, Ariz).

Measurement of CO₂ concentrations is a more practical way to estimate the adequacy of ventilation. CO₂ can be measured with standard indoor air quality (IAQ) monitors (TST Inc, Shoreview, Minn; Enviro-Equipment, Inc, Pineville, NC). CO₂ concentration in an adequately ventilated building should remain near outdoor levels. Increased CO₂ concentrations, particularly if greater than 1000 parts per million, indicate that ventilation is inadequate.²⁸ Low CO₂ measurements might exist in a poorly ventilated building if no occupants are present at the time of sampling.

Air circulation occurs when air moves as a result of air-pressure differences. This movement represents a possible distribution pathway for fungi and their byproducts. Detection of differential pressures with an airflow meter can identify potential routes of dispersal that can be used for subsequent system redesign. A more direct way to detect the direction of air flow is through use of chemical smoke, which can be dispersed and tracked (Gas Detect Smoke Tube; Zefon International, St Petersburg, Fla).

Air sampling

Fungi might cause structural damage without traveling to living areas where occupants reside. For fungal exposure to occur, it is necessary to hypothesize that spores or fungal metabolic products travel from sources to living spaces, if those locations differ. The release of spores into air for subsequent dispersal depends on many factors, including the amount of moisture that is available and the presence of air currents that flow across fungal colonies. Air flowing across actively metabolizing colonies might carry metabolic products, such as micro-

bially generated volatile organic compounds (MVOCs), to living spaces. When moisture is removed from an actively growing fungal colony, the stress might encourage spore production. Air flowing across these colonies might carry spores to living spaces.

The most direct way to identify paths through which spores move is to measure their concentration in air along airflow pathways. This can be done either by means of intermittent “grab” sampling or, if the bioaerosol is suspected to be intermittent, by means of continuous sampling for a period of time sufficient to detect it. Spore counts in a room represent either an equilibrium state or a changing state. Spores traveling down a concentration gradient represent dispersal pathways. Equilibrium spore concentrations depend on their entry and exit rate, local production rates, and the rate at which spores settle out. A single air sample in an environment that is in equilibrium can provide a reasonably accurate estimate of the typical concentration of spores in that room. Such air samples might permit identification of potentially damaging fungal contamination, even when surface colonies are not readily visible.

Air-sampling devices and strategies. Selection of a sampling device and strategy depends on the hypothesis under consideration. The anticipated characteristics of the bioaerosol (expected concentrations and types of spore), the types of analytic tools available, the required detail of the results, and the time frame in which the sampling must be completed all should be considered.^{29,30} Sample volumes and collection times directly affect the lower detection limits, and this affects the accuracy of the measurements.

Certain features are particularly desirable when selecting an air-sampling device. These include the ability to collect over a wide range of times, portability (particularly if a battery option is available), multiple sampling on a single microscope slide, and ease of analysis. Table I lists some of the strengths and weaknesses of various types of sampling devices. These devices do not measure personal exposure to spores but rather the concentration of spores per cubic meter at a single time and location. Total exposure in a location is the integrated spore count over time.

Measurement of total spore concentrations. Determination of total spore concentrations in air is useful for identifying pathways through which spores travel from sources to exposure sites. Slit impaction samplers, such as the Allergenco (Table II), are particularly useful for this purpose. The number of rooms to sample depends on the history of symptoms as stated by building occupants, as well as the expected path from sources to living areas. Spores that might be confined to a crawl space with no route to spread to the living space, for example, are unlikely to cause symptoms, although they might indicate the presence of a problem that will lead to building damage. It might be desirable to collect 3 or more air samples in each room at different times both before and after disturbance of carpeting and room contents to improve the statistical accuracy of air samples.

Results of visual spore counts should be reported in an

interpretable format. The lower level of detection should be provided, and an outdoor reference count should be measured. A ratio of indoor to outdoor counts should be noted next to each genus to facilitate interpretation. This indicates whether a particular spore infiltrated from outside or originated in the building.

Expected spore concentrations in “normal” buildings have been described.^{15,31,32} Because spore concentrations that are likely to cause health effects depend on the length of exposure and individual sensitivity, it is not possible to give a single threshold for such effects. An environmental assessment report therefore should avoid reference to potential health effects of particular counts.

Measurement of viable spore concentrations. Viable spores are collected onto agar by using suction devices. Because desiccation of the culture medium occurs rapidly, short collection times are commonly used, reducing the likelihood of identifying intermittent bioaerosols. Viable spores are collected to determine fungal species, analyze colonies for toxic metabolite and antigen production, and demonstrate the safety of the environment for occupants who might be immunocompromised, such as those in a bone marrow transplant unit. Analysis of viable collections is more laborious, takes longer, and is less representative of the bioaerosol than visual methods. Therefore it should be used only when there is a specific need for the type of information that viable sampling can provide.

Viable spore counts should be reported in CFUs per cubic meter of air. In addition to a total count, the report should state the concentration of each species identified, as well as a comparison with the outdoor count for each spore type. Interpretation of viable colony results must take into account factors that affect growth because many genera of ascospores and basidiospores grow poorly on commonly used media, and rapidly growing genera will tend to overwhelm slowly growing ones. Additionally, each CFU might represent multiple spores or even viable mycelial fragments.

Sample analysis. Air samples might be analyzed by the environmental consultant or they might be sent to a commercial laboratory. Either way, samples should be analyzed by experienced individuals. Although a general impressions can be obtained with a magnification of 400× to fully enumerate the numbers and types of spores, it is best to count at 1000× with oil emersion. Certification of microscopists is provided by the National Allergy Bureau of the American Academy of Allergy, Asthma and Immunology (www.aaaai.org) and by the Pan American Aerobiology Association (www.paaa.org). The American Industrial Hygiene Association certifies laboratories for microbiologic analysis (www.aiha.org).

Features that characterize good laboratories include the following:

- at least 1 year’s experience with the types of samples they analyze;
- availability of quality control data on request and a written quality assurance plan;
- client support, including interpretation of analytic results; and

- participation in at least one of the recognized certification processes (American Academy of Allergy, Asthma and Immunology, Pan American Aerobiology Association, or American Industrial Hygiene Association).

METHODS TO MEASURE FUNGAL EXPOSURE

Identification of fungal contamination in a building can lead to strategies for remediation when there is damage or aesthetic concern. A series of hypotheses leading to health effects requires determination of the extent of exposure. Because personal sampling is not widely available, measurement of fungal substances in house dust might provide an estimate of personal exposure over time.

Collection and analysis of house dust

House dust is a heterogeneous mixture of substances, including emanations from dust mites, cockroaches, pets, and human subjects. It also contains viable and nonviable spores, mycelial fragments, fungal proteins, and byproducts of fungal metabolism. Dust analysis therefore is used to hypothesize about the presence of fungi or fungal byproducts that have accumulated over time in a room. This time-integrated information is complimentary to air-sample data, which represents a specific instant of time.^{24,33} Multiple dust samples might be required from known traffic patterns and various types of upholstered furniture to provide an accurate estimate of total exposure. Suction devices collect settled dust from a measured area, or a sample of dust from the occupant's own vacuum cleaner can be used.

Visual and cultural analysis. Visual analysis of a tape-lift dust sample can provide a semiquantitative determination of the genera and numbers of spores present. Dust also can be cultured for viable fungi by adding a measured amount of dust into a liquid matrix and spreading it on an appropriate agar, giving an estimate of CFUs per gram of dust.

EIA for allergens and antigens. Measurement of dust allergens tests hypotheses about the amount of exposure individuals have to fungal allergens irrespective of whether sources of fungal contamination are still present. An occupant might be exposed to fungi allergens in dust sufficient to cause symptoms, even when few or no airborne spores are present. The characterized method for measuring house dust allergens is with EIAs for specific allergens or for allergenic species.³⁴ Commercial EIAs are available for measurement of cat,³⁵ dust mite,³⁶ cockroach, and rodent (Indoor Biotechnologies, Charlottesville, Va).³⁷ In addition, assays for fungal allergens (Alt a 1 and Asp f 1) are available, as well as assays for whole fungal species.^{24,38} Because fungi are known to have varied expression of allergens, the absence of specific proteins does not necessarily prove the absence of those fungi.

Polyclonal antibody-based assays detect a broader range of fungal antigens; however, they might correlate poorly with the presence of spores. We have demonstrat-

ed that airborne spore counts and dust antigen assays of fungal allergens in dust correlate for certain species, such as *Cladosporium* and *Aspergillus*,³² but they do not correlate well for others, such as *Alternaria* species.³⁸ Polyclonal assays are useful to document the removal of sources of allergenic material.³⁹

Ergosterol and glucans. Although culture and microscopic methods for measuring fungi are useful, each has its limitations. Neither method can be easily automated, and therefore the cost of analysis is likely to remain high. For these reasons, chemical methods for measuring total fungal exposure would be helpful. Two candidate proxies for total fungal biomass under investigation include ergosterol and Beta-1,3-D glucan.⁴⁰ Currently, each substance is difficult to measure,^{41,42} and few studies have been done comparing their concentrations with actual fungal exposure.^{43,44}

Beta-1,3-D glucans are straight or branched chain glucose polymers that are present in most fungi either bound to chitin or on the cell wall as free polymers.⁴⁵ They are potent activators of numerous cells, such as macrophages and neutrophils, in the human immune system.^{46,47} Glucans are related to endotoxins in that they elicit a positive result on the Limulus assay.^{46,47} Because glucans are found in both bacteria and fungi, their presence is not specific for fungal biomass.

Ergosterol is found in the membrane of a majority of fungi but is absent from most other microorganisms.^{16,48} Measurement of ergosterol is difficult to perform and therefore is not performed by most analytic laboratories. As new methods for measurement of ergosterol and glucans are developed, use of these fungal biomarkers might be more widely available.^{42,49}

Mycotoxins. Many genera of fungi have evolved the ability to produce toxic metabolites.^{50,51} Their purpose is to inhibit the growth of competing organisms. The list of known mycotoxins is extensive and ranges from relatively simple sesquiterpenes, such as lemonine, to complex heterocycles, such as cyclosporine. Since their implication in animal diseases in the 1960s, mycotoxins have been the subject of intense scientific interest.^{52,53} Public interest in mycotoxins has increased because of concerns over biologic warfare, the T-2 toxin,^{15,54} and toxins from *Stachybotrys* species.⁵⁵

Mycotoxins are present in relatively small concentration on individual spores, and many species produce mycotoxins with similar structures, making analysis difficult. Identification and measurement of mycotoxins require advanced analytic instrumentation, such as gas chromatography mass spectroscopy or liquid chromatography mass spectroscopy.⁵⁶ Building materials grossly contaminated with fungi, such as *Stachybotrys* species, might produce sufficient quantities of mycotoxin to be measured,^{57,58} and they have been detected in urine of exposed human subjects.⁵⁹

Samples for mycotoxin analysis can be collected from contaminated materials, such as drywall, carpet, or wood, or even from house dust. Recently, an EIA for total tricothecenes has become available (EnviroLogix, Portland, Me). This test is specific for an epitope shared by

many tricothecenes and their intermediate metabolites and breakdown products. The test appears to detect mycotoxins in most samples of house dust (personal observation). The amount and duration of exposure to specific mycotoxins that is necessary to cause health effects remains to be determined.

MVOCs

Fungi produce a complex mixture of low-molecular-weight and high-molecular-weight volatile compounds. Many of these MVOCs have extremely low odor thresholds (1-10 ppt), causing a musty smell that might be noticed by the occupant before significant problems develop.⁶⁰ More than 500 different MVOCs have been identified, including mixtures of alcohols, aldehydes, amines, aromatic and chlorinated hydrocarbons, ketones, sulfur-based compounds, and terpenes. Measurement of MVOCs can be performed to test hypotheses related to the presence of fungi as a source of substances that are aesthetically unpleasant. In most cases MVOCs do not by themselves cause health problems, acting instead as irritants⁶¹; however, most persons find the smell to be repulsive.

MVOC measurement can be used to identify hidden sources of fungal contamination. Sampling methods generally involve capture onto an adsorbent material, followed by desorption and detection in the analytic laboratory. It is also possible to capture a small volume of air in an evacuated container for subsequent injection into the gas chromatography mass spectroscope for chemical analysis. The level of detection decreases as the volume of air sampled increases. Attempts to develop species-specific MVOC profiles have met with some success,⁶² although the sampling and analytic procedures are too expensive for routine use at this time.

Limitations to MVOC analysis are that a single sample represents one point in time that might not represent typical conditions. The actual concentration of MVOCs might vary by orders of magnitude depending on ventilation, substrate moisture levels, availability of food sources, and competing microorganisms. In addition, some volatile organic compounds might originate from nonmicrobial sources.⁶¹

Several new technologies might address these limitations. A portable gas chromatography and/or gas chromatography mass spectroscopy (Inficon, Syracuse, NY) unit now can be carried from room to room, with a sampling probe allowing for real-time gas sampling. With this, the investigator could track MVOCs to source locations. MVOC analysis with these devices can be performed in as little as 3 minutes, although the level of detection generally is not as low as for analyses of larger volumes of air. The zNose (Electronic Sensor Technology, Newbury Park, Calif) is a portable, ultrafast gas chromatography analyzer with a quartz crystal-based acoustic wave interferometer detector that is used to create a reproducible 360° pattern or Vaporprint. Prism Analytical Technologies (PATI, Mt Pleasant, Mich) has developed an ultrahigh sensitivity method by using a specially designed sampler containing multiple matrices.

The MOLDSCAN sampler can detect concentrations as low as 300 ppt.

REPORTING THE RESULTS

Every report should contain information in a format that is useful to the person who needs to interpret the results. Unfortunately, poorly designed reports are common. The following information should be included in environmental reports.

Scope of work

The scope of work should list all hypotheses and a description of the sampling done to test them. It also should discuss the limitations of using single sampling and analysis events.

Site physical description

The physical description should summarize information about the building, including its address, size, age, number of occupants, and the date of the assessment and the weather on that date. Inclusion of a schematic layout of the building is helpful.

Mechanical review

This includes a description of the exterior, including roofing and guttering, foundation, HVAC systems, plumbing, mechanical appliances, and ventilation. Ideally, a picture of each major component should be included along with a description of any abnormalities that might contribute to fungal contamination.

Sample location and circumstances summary

The sample locations and circumstances summary lists the locations where samples were taken, as well as a description of the physical and mechanical attributes of the building. The report should document any special circumstances that accompanied sample collection, such as whether fans were on.

Summary of analytic results and recommendations

Analytic results should be summarized along with a description of findings from the visual walk-through. Interpretation of results and recommendations should be confined to those that are supported directly by data obtained during the assessment.

Analytic results

Analytic results should be included for all samples on which conclusions and recommendations are based. These should include results of IAQ measurements (eg, temperature, humidity, CO₂, CO, SO₂, NO₂, O₃, and volatile organic compounds), including detection limits, recommended exposures when known, and a description of instrumentation. Air samples should include measured spore counts by genus both for rooms sampled indoors and outdoor counts, as well as, for convenience, a ratio of the two.

Surface and bulk sample results should be reported on a semiquantitative scale and should include a photograph of the collection site. Vacuum dust samples should be quantitative whenever possible, with results expressed either in CFUs per gram of dust or per square meter.

Sampling instruments used

The report should also state the instruments used for collecting the sample and what methods or protocols were used. A statement of recent calibration or calibration protocols followed should also be included.

Laboratory reports

Good laboratory reports should include the following: (1) the name of the laboratory and patient-client information, including site address; (2) sample information, including sampling date, time, location, and a unique sample identification number for each sample; and (3) sample results, including all fungi identified, detection limits, the analyst's name, and the date of analysis.

The results of airborne spore concentrations should include an outdoor count. A ratio of indoor to outdoor spore counts for each genus and genus predominance expressed as a percentage of the total can facilitate interpretation.

Indoor air samples, whether viable in CFUs or nonviable in individual spores, should be quantitative, with results usually stated as spores or CFUs per cubic meter of air. Vacuum dust samples should be quantitative whenever possible, with results either per gram of dust or per square meter.

CONCLUSION

The expansion of the fungal detection and remediation industry, as well as the proliferation of fungus-related lawsuits, makes it essential that standardized protocols for environmental assessment be established. Currently, the medical, legal, and insurance institutions and industries cannot proceed in this area because the related science has not yet provided sufficient clarity. As the tools of testing and investigation improve and the scientific and medical pursuits coalesce, we expect the industry to mature. Only then can existing and emerging questions about contamination and the health effects of indoor fungi be answered. Certainly there is an urgent need for investigators to determine the extent and mechanisms of health effects caused by fungi and, if present, to establish exposure thresholds and guidelines for the medical community. The critical step at this point, however, is to create a foundation on which further standardization and investigation can evolve, and that is what we have attempted here by describing and proposing the above investigative strategies and guidelines.

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TABLE E1. Healthy homes checklist

For each of these items, indicate yes or no.

Health symptoms

Do you have any of the following symptoms when at home, and that seem to get better when you leave?

1. Headaches?
2. Itchy, watery, or burning eyes?
3. Breathing problems, coughing, or shortness of breath?
4. Nasal congestion?
5. Dry, sore throat?
6. Drowsiness?
7. Skin rashes?
8. Fatigue?
9. Difficulty concentrating?
10. Feelings of dizziness or confusion?
11. Increased allergies or respiratory diseases?
12. Increased asthma attacks?
13. Do your symptoms get better when away from certain rooms in the home?
14. Do your symptoms get worse at night or on weekends?
15. Are your symptoms worse in the morning and get better during the day?
16. Does anyone else in the home have symptoms when at home?
17. Is anyone in your family particularly sensitive to chemicals?
18. Does anyone in the household have allergies, asthma, bronchitis, emphysema, or heart problems?
19. Do you cough, wheeze, have chest tightness, or feel short of breath year round?
20. Do your symptoms get worse in the spring or fall?

Home assessment

21. Can you see dust or dirt on your furniture, walls, ceiling, and curtains?
22. Do you vacuum less than once a week?
23. Do you have wall-to-wall carpeting in your kitchen or bathrooms?
24. Do you have wall-to-wall carpeting in more than half of the rooms in your home?
25. Have any recent changes been made to the home (painting, remodeling, new items purchased)?
26. Have you ever had any problems with mice or rats in your home?
27. Have you ever had any problems with cockroaches, ants, or other insects in your home?
28. Do you have, or have you ever had, any furry and/or feathered pets?
29. Does your air conditioner ever leak water onto walls or carpeting?
30. Does moisture regularly build up on your windows or walls?
31. Is any part of your living area under ground, and if so, does this area ever get wet and/or stay wet for long periods (more than 1 week)?
32. Has water entered your home through roof, window, or plumbing leaks?
33. Does your home have mold growing anywhere other than the shower?

Indoor air quality

34. Does anyone smoke inside your home?
 35. Does any part of your home seem extremely cold or hot?
 36. Are all your windows sealed shut or do not open?
 37. Does your home sometimes smell “stuffy,” “stale,” or “musty”?
 38. Is a gas stove, kerosene, or oil heater used without ventilation in your home?
 39. Has it been 6 months or longer since your furnace filters were changed?
 40. Is a fireplace or wood-burning stove used regularly in your home?
 41. Are household chemicals or sprays (eg, cleansers, cleaning sprays, scrubbers, oven sprays, or air fresheners) used regularly for household cleaning?
 42. Does anyone use chemical pesticides (eg, bug sprays, plant sprays, or rat poison) inside your home for any reason?
 43. Do any family members have hobbies that require chemicals (eg, paints, solvents, glues, strippers, or varnishes) or create dust?
 44. Are there any activities in your home that generate odors, gases, or strong fumes?
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