

Fungal Sampling Methods

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General Considerations

Currently, the method of choice for assessing potential exposures to airborne molds and mycotoxins in indoor environments involves the collection and identification of fungal propagules. Determining types and prevalence of various species of fungi present on surfaces and in the air allows for assessment of active growth within buildings. In many cases, the underlying question is simply “Is active mold growth occurring indoors”? A genus level identification is sufficient in most cases, but for particular cases where correlations are being made to health effects it may be necessary to have all fungi identified to species. In groups that commonly produce secondary metabolites, such as *Penicillium* or *Aspergillus*, the type, quantity, and toxicity of these compounds varies considerably among the species. Indoor sampling protocols should involve a variety of sample types to get a well-rounded assessment. Areas of visible mold may be sampled directly by sending bulk material or tape samples for identification and settled dust may be collected for analysis. Sampling should include air monitoring in selected problem and non-problem areas with an outdoor comparison sample. Culturable and non-culturable methods have differing pros or cons. Although either method will usually detect major problems, a combination of the two provides for the most reliable interpretation.

Non-Culturable Bioaerosol (Spore-trap)

Non-culturable (also known as “non-viable”) air samples are collected using a variety of spore-trap samplers. Air is drawn across an adhesive, impacting and trapping all particulate matter in the air, including fungal spores. Some sample collection devices utilize adhesive-coated glass microscope slides, including the Burkard, Allergenco MK-3, and BioSIS 2000. Other disposable sampling types include the Air-O-Cell® cassettes using the Zefon Mini-Pump, and the Cyclex-d™ or Micro5 cassettes. Spore-trap samplers have demonstrated an excellent ability to allow sensitive detection of *Stachybotrys* spores present in low levels. They allow for rapid analysis when required and adequately determine the levels and proportions of various spore types determined to genus or broad category. Although they lack some specificity in identification, they recover types of spores that do not grow or compete well in culture. They have the additional advantage of collecting all airborne particles for microscopic observation. Elements such as pollen, insect parts, mites, epithelial cells, fiber glass and carbonaceous debris may be detected to further broaden the scope of the IAQ investigation. An example of a sampling protocol using the Air-O-Cell® cassette is provided below: The Zefon pump is calibrated to a flow rate of 15 liters/minute (Lpm) using a factory-supplied rotameter (annually calibrated by the manufacturer). The tape seal on the cassette inlet and outlet is removed, and the outlet is connected directly to the Mini-Pump port. Make sure the rectangular orifice with a slit inlet is facing outwards on the sampler. Per manufacturers recommendations, each sample is collected for 5 minutes under normal building and outdoor conditions. If the area is very clean (e.g., environmentally controlled office building) with little airborne

dust, the sampling time may be extended to 10 minutes. Likewise, when the environment is highly contaminated or dusty, the sampling time should be reduced accordingly. After sampling is completed, replace the seals over the cassette inlet and outlet. The sample is labeled in conjunction with the sample number and information on the chain of custody (COC) form. Remember to include the sampling time and flow rate (or total sample volume) on the COC so calculations of the total spores/m³ can be made. Samples will not deteriorate in transit and may be shipped at room temperature to the laboratory at your convenience.

Culturable Bioaerosol

Culturable (also known as “viable”) air samples are collected on agar culture media. One of the most common types of samplers are the vacuum pump sieve-impaction samplers such as the Andersen N6, a high-volume vacuum pump calibrated to a flow rate of 28.3 liters per minute (Lpm) using a factory-supplied rotameter (annually calibrated by the manufacturer). Other sieve impactors have higher flow rates, including the Surface Air System (SAS Super 100), Millipore MAirT, and the EM Science MAS 100. These samplers employ standard 100 mm Petri dish plates or contact plates for impaction. Alternatively, The Biotest RCS is a centrifugal sampler that uses agar strips for culturable sample collection. Once at the lab, all of these sample types are incubated to induce the impacted fungal spores to grow, allowing identification on both a genus and species level. Sampling recommendations for fungal bioaerosols are consistent with the American Industrial Hygiene Association *Field Guide for the Determination of Biological Contaminants in Environmental Samples* (AIHA, 1996) as well as the manufacturers’ instructions for each sampling device utilized. The limitations of interpreting results obtained from these sampling methodologies are described therein. The primary advantage of culturable bioaerosol sampling is that precise identifications are possible, crucial for species ID of *Penicillium* and *Aspergillus*, and important for the recovery and recognition of a wide variety of potentially toxigenic molds such as *Paecilomyces*, *Fusarium*, *Trichoderma*, *Phoma*, *Acremonium*, and *Wallemia*. An example of a sampling protocol using the Andersen-N6 sampler is provided below. Before each sample is collected, the sampler housing (orifice and base) should be disinfected and cleaned with 70% alcohol and allowed to air dry immediately before the culture plate is placed inside. The sampler should be positioned such that the inlet is facing directly upward at a height corresponding roughly to sitting or standing breathing zones. Remove the cover of the agar plate and insert on the base of the N6. The inlet cone is placed on top of the perforated plate and the entire assembly is sealed using the connecting clamps. A variety of media can be used for sampling depending on the strategy and sampling conditions. Generally for routine analysis of a wide spectrum of fungal species, malt extract agar (MEA) and/or potato dextrose agar (PDA) amended with antibiotics are recommended. Dichloran glycerol 18% agar (DG18) is used to recover some xerophilic (dry tolerant) fungi such as certain *Aspergillus*, *Penicillium* and *Wallemia*. Cellulose agar (CEL) is useful to detect cellulolytic fungi such as *Stachybotrys*, *Chaetomium* and *Alternaria*. Per manufacturers recommendations, each sample should be collected for 2-3 minutes (unless very clean or highly contaminated conditions prevail). After sampling is completed, the cover is replaced and the culture plate is sealed with laboratory film. The collection plate is labeled in conjunction with the sample number and information on the COC form. Remember to record the sampling time and flow rate (or total sample volume) on the COC for calculations of the total CFU/m³. Samples are perishable and should be shipped via overnight courier to the laboratory. If sample storage is required prior to shipping, they should be refrigerated. Plates may be transported in insulated coolers (with or without cold packs depending on conditions

expected during shipping) to protect samples from environmental fluctuations during periods when samples might be expected to experience temperature extremes.

Surface Tape (Microscopy)

If you see visible or suspected fungal growth on dry, hard surfaces, the quickest and most cost effective sampling method to confirm mold growth is to sample with a piece of clear tape. Use a piece of Scotch Transparent Tape about 1.5 inches long. Fold one end over to provide a 'handle' and press the sticky surface using light pressure against the area in question; do not vigorously rub back and forth or use excessive pressure. Peel off the tape immediately, and make sure some of the surface material is adhering to the tape. Do not fold the tape on itself, but rather, place the sample inside a clean freezer-strength Zip Lock® bag, or other similar bag, or mount it on a glass microscope slide (ensure slides are adequately protected to prevent breakage in transport). The sample is labeled in conjunction with the sample number and information on the COC form. Samples will not deteriorate in transit and may be shipped at room temperature to the laboratory at your convenience. Unfortunately, one limitation of tape samples is that they are not suitable for culturable analysis.

Bulk Samples (Microscopy and Culturable Fungi)

Any small pieces of building material (e.g., drywall, carpet, baseboard, tack strip, insulation) or contents (e.g., furniture, drapes, clothing, paper) with visible or suspected fungal growth can be collected for analysis. The samples should be sealed into a clean Zip Lock® bag or other container. Water samples should be collected in tightly sealing, sterile, plastic containers. The sample is labeled in conjunction with the sample number and information on the COC form. If culturable analysis is requested, or if the sample is perishable, the package should be shipped via overnight courier. Wet samples should be transported in insulated coolers with cold packs to prevent proliferation of organisms during transit.

Dust (Culturable Fungi)

Settled dust can be collected directly from hard surfaces or extracted from carpeting and other porous surfaces (e.g., furniture, clothing, books and papers) using a 0.45 μ m methyl cellulose ester (MCE) filter dust cassette and collected with a vacuum pump. The caps on the cassette inlet and outlet are removed and the outlet can be attached to the pump via 2 feet of plastic tubing. Although the samples may be collected for a variable length of time and over a variable area in accordance with the desired sampling goal and dust content of the surface being sampled, a standard pattern of collection helps to provide uniformity and comparability in sample results. For example, to collect carpet dust, go to a low traffic area and sample using a standard time, flow rate, and surface area (e.g., 2 min., 28.3 Lpm, 1 m²), while vacuuming the surface horizontally and then vertically to cover the entire area. After sampling is completed, replace the caps over the cassette inlet and outlet. The cassette is labeled in conjunction with the sample number and information on the COC form. Samples will not deteriorate in transit and may be shipped to the laboratory at your convenience.

Surface Swab (Culturable Fungi)

Visible or suspected fungal growth on hard surfaces can be sampled using a sterile swab (e.g., BBL CultureSwab) for culturable analysis. The sterile swab is removed from its packaging and can be moistened with the holding medium in the tube prior to use on dry surfaces. The swab is then placed onto the area of concern and rolled to allow a sufficient amount of material to accumulate on the swab tip. The swab is then inserted into the transport medium tube and tightly sealed. To provide a quantitative assessment of specific areas, a template of 1 to 100 cm² is used while swabbing the surface horizontally and then vertically to cover the entire area. The swab may also be used for non-culturable microscopic examination, but the tape sample is more appropriate for this type of analysis in most cases. Swabs are especially useful for wet surfaces where material will not adhere adequately to the tape or in hard to access areas. The swab is labeled in conjunction with the sample number and information on the COC form. Samples are perishable and should be shipped via overnight courier to the laboratory. Samples requiring enumeration of bacteria or yeasts should be shipped in an insulated cooler with cold pack to minimize growth in transit.

Other Microbiological Sampling Methods

Bacterial Sampling

In general, sample for bacterial contamination following the same guidelines as for the fungi. Some unique situations and differences in strategy include:

- Bacteria are not easily detected by microscopic examination, so culturable analyses should be conducted.
- Airborne bacteria can be collected on tryptic soy agar (TSA)(recommended for a wide spectrum of environmental bacteria).
- Airborne *E. coli*, coliforms, and other gram-negative bacteria can be identified using MacConkey agar (MAC).
- Airborne *Legionella* can only be detected using highly selective agar media (e.g., BCYE).
- If contamination by thermophilic actinomycetes is suspected, use TSA.
- Swabs of humidifiers, air conditioners, machine fluids and other wet sites are an excellent means of assessing overall bacterial contamination.
- Use of sterile, rayon-fiber tipped swabs with holding medium (e.g., BBL CultureSwab) is critical for optimal bacterial recovery. Naturally occurring antimicrobial agents found in cotton may be detrimental to recovery of bacterial contaminants.
- Swab and bulk materials for bacterial analysis should be sent to the laboratory in insulated coolers with a cold pack to minimize growth in transit.