



RECOMMENDATIONS FOR SAMPLING FUNGI IN INDOOR ENVIRONMENTS

Investigation of fungal contamination in indoor environments normally includes visual inspection and sampling. The sampling strategy and sampling methods should depend on the goal of the investigation. This document is intended to provide basic recommendations for common sampling methods.

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1.0 AIR SAMPLING

Collection of air samples for indoor mold investigations is done to detect and quantify mold spores and other allergens in the air. Air sampling aids in the assessment of possible human health effects from exposure to these organisms, as well as in monitoring the effectiveness of control measures.

Air samples may be analyzed by various methods, including direct microscopy, the culture method, and quantitative polymerase chain reaction (QPCR). Direct microscopy is particularly designed to detect bioaerosols present in the air regardless of their viability. Particles that can be found in air samples include fungal spores, pollen, and other fragments of organisms such as plants, insects, animals, etc., or particles of inorganic origin such as building materials.

Sampling Recommendations

The number of samples per area may vary with the specific case, but enough samples should be collected to draw conclusions that are scientifically sound and legally defensible. In many cases where health issues are involved, larger numbers of samples, or re-sampling, may be required to obtain statistically sound results.

- Always include samples of the outdoor environment for comparison.
- It is recommended that samples of a non-problem area be taken for comparison.
- Blank cassettes/plates are recommended on a regular basis. These cassettes/plates should be open in the testing area, in exactly the same fashion as regular samples, but no air will be drawn through them.

- Overexposure in the cassettes is indicated when the slide in the cassette is completely opaque or white. In this case, another sample must be taken using less time.
- Make sure that your rotameter or primary calibration device is working properly. Correct calibration of the pump is imperative to obtain good samples.
- Never use plates or cassettes that appear opened or damaged.
- The use of protective equipment such as a mask, gloves, and protective clothing is recommended in highly contaminated environments, or by sensitive individuals.

1.1 Air Sampling for Fungal Direct Examination

For direct microscopy, air sampling is performed using a specific particulate capture device. The most commonly used devices are spore trap cassettes. This discussion is primarily based on Air-O-Cell[®] cassettes, though other cassettes may use similar mechanisms. The Air-O-Cell[®] cassette is designed for rapid collection of a wide range of airborne particulates based on the principle of inertial impaction. Particulate-laden air is accelerated as it is drawn through the cassette's tapered inlet slit, and directed toward a small slide containing a sticky collection media, where the particles become impacted, and the air flow continues out the exit orifice.

The adhesive nature of the collection media prevents the collected particulate from blurring or being washed away during the staining process, and it also eliminates sample loss from vibration during handling and shipment. After sampling is completed, the cassettes are sent to a laboratory, where the slides are removed, and direct microscopic analysis can be immediately performed.

The advantages of this method include:

- Rapid turnaround times; results can generally be obtained in 24 hours or less.
- The method facilitates determination of the total number of bioaerosols present in the testing area, including viable and non-viable. Since many health parameters, such as allergenicity, do not require the bioaerosol be viable, the results with this method are more indicative of the actual exposure than those obtained with culture-based systems.
- Partial identification of the types of spores present in the sample.

The disadvantages of this method are:

- Genus identification and/or species identification is mostly not possible.
- Total numbers are reported, including both viable and non-viable spores.

As with any sampling method for indoor environments, it is not recommended that a single method be utilized to draw conclusions in an indoor mold investigation. A combination of indoor sampling methods, including direct microscopy, viable, surface samples, etc., is recommended in order to obtain a better investigative profile.

Materials

- Spore trap cassettes (Air-O-Cell[®] or other brand). This discussion uses Air-O-Cell[®] for illustration purposes. For other types of spore trap cassettes, the reader is encouraged to follow the manufacturer's recommendations.
- Vacuum pump capable of pulling at least 15 Liters per minute (L/m) with flexible tubing.
- Calibrated rotameter to calibrate the pump; or, in sensitive cases, a primary pump calibration device such as a DryCal flowmeter (BIOS International Corp., Butler, NJ).

Procedure

1. Calibrate the vacuum pump at 15 L/min. The cassettes are designed to work best at this flow rate. Higher flow rates may cause bridging of the filter and distortion of spores, whereas lower rates may affect the ability to collect all spore types in a uniform pattern.
2. Remove (but do not discard) the tape covers from both the inlet and outlet of the cassette.
3. Insert the tubing in the outlet of the cassette, and direct the inlet to the area to be sampled.
4. Turn on the pump and take sample for the desired time.
5. Duration of sampling will vary, depending on the environment to be sampled. Recommendations of some manufacturers are presented in the table below.

Recommended sampling intervals

<i>Environmental Conditions</i>	<i>Sampling time (minutes)</i>
Outdoor sampling on a clean, windless day	10-60
Clean office environment	10
Indoor environment, high activity of personnel	5
Indoor environment, evidence of drywall renovation, or industrial dust.	1
Indoor environment, visible dust emissions from point sources present	0.5

6. Weather and/or geographic location impact the duration of sampling, especially in outdoor environments. In some cases where large investigations are in process, it may be prudent to conduct preliminary screens to determine the optimal collection times.

7. Remove cassette from tubing and replace the tapes in both ends of cassette. Label the cassette.
8. Fill out all the information in the Chain of Custody, including sampling duration.
9. Ship samples and paperwork overnight to Aemtek, Inc. No ice is necessary to ship Air-O-Cell samples. However, packing or cushioning material is recommended to avoid possible breakage of the cassettes.

1.2 Air Sampling for Culture-based Analysis

Information on the presence of culturable molds may be a possible indication of active growth in indoor environments. Active growth in indoor environments needs to be addressed, and the source needs to be detected immediately to stop further contamination. The presence of culturable molds in indoor environments implies not only the presence of allergens and mycotoxins, but potentially also pathogens that may cause disease, especially in people with compromised immune systems.

Detection of culturable organisms in the air is performed by impacting air samples directly onto culture media. Unlike direct microscopy, this method provides quantitative and qualitative data on the specific genera and/or species present in the indoor air.

This method is usually performed using a vacuum pump and an Andersen N-6 single-stage viable impactor (or equivalent). Air is drawn through the pump and, by the inertia principle, particles are impacted onto Petri dishes containing specific agar. The type of media used in the Petri dishes varies depending on the purpose of the investigation (mold, bacteria, or specific genera). The agar plates are sent to the laboratory, where they are incubated for a specified period, usually 7-10 days for mold species. At the end of this period, laboratory analysts quantify and identify (to the species level, if so desired) most organisms that have grown on the media.

As with other sampling methods for indoor environments, it is not recommended that this procedure be utilized as the sole basis for drawing conclusions in any indoor bioaerosol investigation. A combination of different sampling methods is advised, to obtain a more comprehensive investigation.

Some advantages of this technique are:

- The ability to obtain genera and species identification of the organisms; for example, even the most significant genera such as *Aspergillus* and *Penicillium* cannot be differentiated on spore-trap samples, while differentiating species can be performed on cultures.
- The presence of viable or culturable organisms in the air may indicate actively growing organisms in the testing area.
- Culture-based methods can provide quantitative data on viable and culturable fungi.
- Culture-based analysis is one of the most economical ways to identify molds to the species level, especially compared to existing molecular methods.

Some disadvantages of this methodology are:

- Long turnaround times. It takes 7-10 days for the mold spores to grow on the media.
- It detects only culturable organisms, missing all the non-viable organisms that can be allergenic, oxygenic, and/or irritants.
- Total numbers may be misleading, since fast growers may overgrow the medium and mask the presence of slow-growing organisms.
- The technique will also miss those organisms that do not grow on culture media, or that may need a specific medium.

Materials

- Vacuum pump capable of pulling up to 28.3 Liters per minute (L/m).
- Flexible tubing.
- Calibrated rotameter to calibrate the pump; or, in sensitive cases, a primary pump calibration device such as a DryCal flowmeter (BIOS International Corp., Butler, NJ).
- Andersen N6 single stage impactor (or equivalent).
- Petri dishes with culture media. (Media type will depend on the type of sampling and the objective of the sampling. Different media are recommended for different target organisms.)
- 70% alcohol and cotton balls or alcohol wipes.

Procedure

1. Agar plates should be stored refrigerated or in a cool dry place until used.
2. Prior to the test, take plates out and allow them reach room temperature before use.
3. Calibrate the flow rate of the pump with the rotameter or primary calibration device.
4. Attach one end of tubing to the intake of the pump, and the other end to the inlet of the sampler.
5. Turn on the pump and make sure flow rate is at 28.3 L/m. Allow it to equilibrate for 1-2 minutes. Turn pump off.
6. Disassemble sampler by unscrewing the knobs. Avoid touching the inside of sampler. Use of gloves is recommended.
7. Disinfect sampler with 70% ethanol (or equivalent) and let dry.
8. Label Petri dish on the back of the plate, with enough information to identify the plate. The bottom of the plate is the one with medium in it. Use permanent marker.
9. Open the Petri dish and place lid in a sterile bag (Ziplock bags are OK), and keep it there until the sample has been taken.
10. Place the other half (with medium) of the plate in sampler.

11. Reassemble Anderson N-6 sampler and take air sample. Duration may vary depending on the environment (in most cases, from 1-10 minutes). Make sure the duration of sampling is recorded in order to obtain quantitative data in cfu/Liter.
12. Once sampling is complete, disassemble sampler and take plate out of sampler. Close plate immediately with the lid. Be careful to avoid any contamination.
13. Secure the lid on plates with tape; if possible, seal the whole plate with the tape, or add several pieces of tape to keep the plate closed. Place the plate back into the Ziplock bag and make sure labeling on plate is correct.
14. Fill out the Chain of Custody and pack plates for overnight shipping
15. Use wrapping material all around plates to avoid breakage of plates during transportation.
16. Plates need to be kept cold (use ice packs) during the transport. **Do not use dry ice – plates must stay cold but not freeze.**

1.3 Air Sampling for Quantitative Polymerase Chain Reaction (QPCR)

Identification and quantification of mold spores in air can also be determined by QPCR. This method is a DNA-based technology; air samples are analyzed for a specific group of organisms.

This method detects and quantifies viable and non-viable organisms. Sampling is done using a three-piece PCR cassette. This cassette is designed to capture large volumes of air, since overcrowding is rarely a problem for QPCR.

Some of the advantages of this method are:

- Can accommodate large-volume samples, a distinctive advantage for minimizing sample variations and increasing sensitivity.
- Quick turnaround time, 24 hrs or less.
- Highly sensitive.
- Detect total bioaerosols, both viable and non-viable.
- Results are quantitative and qualitative to the species level.

Some disadvantages of this methodology are:

- Limited to the availability of assays (species); may miss species that are not included in the test.
- Doesn't differentiate between viable and non-viable (pathogens need to be viable to cause disease).
- Cost.

Materials

- Vacuum pump with flexible tubing.

- Calibrated rotameter to calibrate the pump; or, in sensitive cases, a primary pump calibration device such as a DryCal flowmeter (BIOS International Corp., Butler, NJ).
- PCR cassette (3-piece, 37 mm cassette preloaded with a polycarbonate filter (pore size of 0.45 µm or 1.0 µm or equivalent)).

Procedure

1. Calibrate pump. Recommended air flow for PCR sampling is between 3-10 L/minute.
2. Remove the red cap (outlet) from cassette and connect it to pump.
3. Remove the blue cap (inlet) from cassette and direct it toward the sample site.
4. Take the air sample. Sampling time is theoretically almost unlimited, 500-3000 liters of air or sampling for over eight hours is the general recommendation.
5. In dusty or other high-particulate environments, lesser volumes are recommended to avoid clogging and possible interference from the dust or particulate.
6. At the end of the sampling time, replace both caps in the cassette.
7. Label each cassette properly. In the Chain of Custody, record the amount of air collected.
8. A blank cassette is recommended with every project as a control.
9. Pack and ship cassettes to the laboratory to be analyzed. No ice is necessary. Packing material is recommended to avoid damage during transportation.
10. Make sure to specify the fungus or group (package) of fungi to be tested for in the analysis.

2.0 SWAB, BULK, AND DUST SAMPLING

2.1 Swab Samples

Swab sampling in IAQ investigation is usually done in combination with other methods, such as air and bulk sampling. It is normally done when the surface is a non-porous (smooth) material, and cutting it is undesirable or not an option (countertops, poles, floors, etc.). Swab sampling is most commonly done by wiping or swabbing a designated area. The analysis of surface samples can provide information about whether a suspect area is supporting microbial growth as a potential source of biological agents in the air. In addition, settled spores can be determined on surfaces. Surface samples can be analyzed to semi-quantify and identify organisms by direct microscopy, QPCR, and/or culture methods.

Materials

Sterile swab (in sterile buffer), or sterile wipe.

Procedure

1. Select the area to sample. It should be relatively non-porous material.
2. With a moist, sterile swab, swab the desired area thoroughly with a rolling motion. Place swab in corresponding tube (zip lock bag are OK), and make sure it is completely sealed.

3. A sterile wipe can also be used to obtain the sample.
4. Each sample must be in an individual bag/tube.
5. If the swab/wipe sample is intended for culture-based analysis, then a sterile sampler should be used, which can be obtained through the laboratory.
6. Label the sample and record the area sampled in the Chain of Custody. Make sure to specify the type of analysis needed.
7. Pack the sample with wrapping material and ice. Ship the sample *overnight* to Aemtek, Inc.

2.2 Bulk Samples

Bulk samples are portions of materials (e.g., pieces of wallboard, duct lining, carpet, etc.) that are tested to determine if they may contain, or be contaminated with, microbial agents. Analysis of bulk samples can provide information regarding possible sources of microbes and their toxins, allergens, and irritants. These analyses can also provide information on the effectiveness of the remediation process. Bulk samples can be analyzed for mold quantification and identification by direct microscopy, QPCR, and culture. They can also be analyzed for quantification and identification of bacteria by culture methods.

Bulk samples may look completely clean, show some discoloration, or clearly have microbial growth.

Procedure

1. Obtain a piece (or pieces) of the desired material to be tested, and place them *individually* in sealable plastic bags (Ziplock bags work very well). The size of the sample will vary, and some criteria should be taken into consideration.
2. The sample should be small so that it can be easily packaged and transported to the laboratory.
3. If the material shows different tones of discoloration or morphology, it is recommended to send either a sufficiently large piece to represent the damage, or take several samples for a full representation of the different bacteria or fungi possibly present in the damaged area.
4. Label each sample properly and record in the Chain of Custody the type of test required.
5. Pack sample(s) with wrapping material and ice (if it is to be cultured), and send *overnight* to Aemtek Inc.

2.3 Dust Samples

Dust samples can be analyzed by direct microscopy, but they are best analyzed by culture-based methods or QPCR.

Material

Standard 3-piece 25 mm cassettes containing 0.8 um mixed cellulose ester (MCE) filters supported on cellulose pads. Some investigators use 37 mm cassettes, which are similar to the 25 mm cassettes.

Procedure

1. The blue plug is the inlet, and the red plug is the outlet. Remove both plugs of the cassette. Attach a small piece of tube to the inlet to collect dust. For better collecting, cut a 45-degree angle at the end of the tube.
2. Connect the outlet of the cassette to a vacuum pump.
3. Airflow rate can be 10-20 lpm for 25 mm cassettes, or 20-25 lpm for the 37 mm cassettes, calibrated using a direct-reading 60 mm rotameter with a range of 0-30 lpm. The investigator can determine how much dust to collect or how large an area is to be sampled.
4. Collect by marking an area of carpet, then brushing the sampler inlet across the surface of the carpet in a horizontal and then vertical pattern.
5. Samples can be collected as variable-area or variable-weight. According to the investigator's preference, the results can be reported on a weight basis as colony-forming units per gram of dust (cfu/g) or, if the sample areas are known to the laboratory, on an area basis as cfu/cm² or cfu/100 cm².
6. Ship the samples to the laboratory. No refrigeration is needed.

2.4 Tape-lift Sample

The primary purpose of the tape-lift sampling method is to confirm the existence of mold growth and to identify the type of fungi at the sampling site, referred to as source sampling. The secondary purpose is to obtain fungal spore distribution data to determine if the sampling site is normal or contaminated (referred to as content sampling). Tape-lift sampling is best used in conjunction with air sampling for verification of indoor mold growth by direct fungal examination. It is also the simplest method of source sampling.

Compared with swab sampling, tape-lift sampling has the following advantages:

- Better preservation of fungal structure, which may allow for identification of higher resolution.
- Does not permit fungal spore germination and yeast reproduction during storing and shipping.

However, tape-lift samples are not suitable for culturable analysis.

Material

Clear, completely transparent, one-sided Scotch tape.

Procedure

1. Discard the first 1-2 inches of tape. Take a new piece of tape, about 2 inches in length, apply the sticky side to the target site, gently press the middle portion of the tape to sample an area of 1 cm² to 1 in², and lift the tape.
2. Put the tape onto the slide, sample/sticky-side down, and secure both ends.
3. Clearly label the slide, put it inside of an individual slide mailer, and send it to the lab.

3.0 HOW TO TRANSPORT SAMPLES

To ensure the quality of your data, the samples should be transported to the laboratory as soon as possible and in a non-compromising way. The goal is to maintain the integrity of the samples and to avoid artificial factors that may impact the credibility of your data. For example, extreme temperatures during sample transportation, or prolonged holding time, may change the population of the microorganisms to be tested. We hope you will find the following guidelines useful, whether you are a local client and prefer to drop off samples at the lab, or if you will ship samples by express carrier.

1. All samples should be adequately packed to avoid leakage of liquid, breakage of slides, or other physical damage to the contents. Culture plates should be individually sealed to prevent contamination.
2. Samples for direct microscopic examination only (e.g., spore trap, tape, and bulk samples) can be transported at ambient temperature of 40-90°F.
3. Cultures and samples for culture-based analysis (e.g., viable fungi, bacteria, water, and sewage screen samples) should be transported to the laboratory as soon as possible after collection, or kept at 4°C for no more than 24 hours before shipping. These samples should be packed with artificial ice packs and/or in coolers to avoid extreme temperature during shipping.
4. If the culture samples are to be shipped on Friday, make sure to mark the packaging **conspicuously** "For Saturday Delivery" and notify the lab for Saturday receiving.
5. Aemtek reserves the right to reject samples if we feel that the quality of our analytical data may be compromised due to the condition of the samples upon receiving.

4.0 HOW TO SUBMIT SAMPLES

To ensure sample integrity and analytical quality, clients are advised to adhere to the following guidelines when submitting samples. Please feel free to call the lab at 510-979-1979 for any additional information.

1. All samples must be accompanied by proper documentation. Use your own sample submission form, or download a Chain of Custody form from the Service section of www.aemtek.com
2. All samples must be clearly labeled and identifiable with the information provided on the Chain of Custody form. Please specify sample volume, analysis requested, turnaround time desired, and preference for reporting.
3. Samples should be individually sealed and properly separated to avoid mixing of samples or cross-contamination.
4. Samples should be adequately packed to avoid leakage of liquid, breakage of slides, or other physical damage to the contents.
5. Samples for direct microscopic examination (e.g., Air-O-Cell, tape, bulk, etc.) can be shipped at non-extreme ambient temperature.
6. Cultures and samples for culture-based analysis should be shipped overnight and packed with artificial ice packs and/or in coolers to avoid high temperature during shipping. See "How to Transport Samples" above for more information.
7. Some sampling supplies, such as swabs and media, are provided free of charge if returned to the lab for analysis. Please contact the lab in advance for sampling supplies.
8. Clients are encouraged to contact Aemtek analysts to discuss any special sampling and sample submission procedures.

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