FAQs About Spore Trap Air Sampling for Mold for Direct Examination

Mold Analysis Document

Developed by the AIHA Indoor Environmental Quality Committee,
AIHA Biosafety and Environmental Microbiology Commmittee, and
AIHA Sampling and Laboratory Analysis Commmittee

Approved by AIHA Board: May 16, 2019

Industrial hygiene investigations for mold in indoor environments may include the collection of air samples for direct examination for fungi, culturable fungal air samples, or both. Such sampling requires clearly defined goals and a sampling plan before sample collection.

Prior to collecting any air samples for mold spores, the indoor environmental quality (IEQ) professional must determine the purpose and relevance of the sampling as well as ascertain the questions the sampling will answer. Air sampling should be considered as a screening tool or as ancillary to an informed inspection. Testing results should confirm observations or otherwise support conclusions made based on the informed inspection.

In the absence of an informed inspection, air sampling alone cannot support any definitive conclusions. Air sampling for mold spores does not and cannot evaluate potential health risks.

Q: What do the terms "viable," "nonviable," "direct examination" and "culturable sampling" mean?

A: Direct examination samples for mold are commonly referred to as "nonviable," "spore trap" or "total spore count" samples for mold or fungi. The term "nonviable" means that cultures are not grown in the laboratory to identify the fungi detected in these samples. These samples are typically collected using an inertial impactor with air sampling cassettes. Some commonly used cassettes include, but are not limited to, Air-O-Cell®, Allergenco-D and Cyclex-D (see note).

When analyzing direct examination samples, fungi are identified using microscopical techniques to examine spores, hyphae and other fungal structures captured by the air sampling cassette. Fungi may be identified to the genus level by direct exam. However, because differentiation of spores by microscopical exam alone can be difficult, fungi sometimes can be reported only as a group (for example, "*Penicillium/Aspergillus*-like" or "type-genera"). In fact, identifying individual species from *Stachybotrys, Aspergillus* or any other genus by microscopical evaluation is impractical without further culturing the sample or using molecular methods.

Results are typically reported in spores per cubic meter of air (spores/m3); the number and/or relative percentage of each spore type in a sample is also usually reported. Common turnaround time for direct exam/spore trap samples is 24 to 72 hours.

Viable mold sampling is more appropriately called "culture-based analysis" for mold or fungi. Types of fungi in air samples are identified by impaction directly onto growth media and by growth of the fungal cultures on the media in the laboratory. Culture-based air samples for mold are commonly collected by inertial impaction samplers. Examples of such samplers include Andersen N6, SAS Super 180, SKC BioStage and Buck BioAire (see note). The sample collected on the impaction surface is incubated in the laboratory. The fungal colonies able to grow on the media are counted and identified by traditional microbiological methods (colony morphology, microscopical examination of spores and hyphae, colony growth characteristics, etc.).

Results are reported as colony forming units per cubic meter of air (CFU/m^3). Turnaround time (meaning the time it takes to provide results after the lab receives the samples) for culturable fungal air samples is usually seven to 14 days. Fungi are usually identified to the genus level, and sometimes to the genus and species level. The number and/or relative percentage of each fungal type in a sample is usually reported as well. Typically, fungal species identification (if offered by the laboratory) involves extra days or weeks of analysis to determine other growth characteristics, and this usually involves extra cost to the customer.

Both direct examination and culturable approaches typically involve collecting and comparing indoor versus outdoor samples. Based on on-site environmental conditions, the investigator is usually trying to determine whether any significantly elevated fungal levels are occurring indoors that are different or unusual when compared with the

outdoor microbial flora. Many investigations also compare levels of fungi in complaint/concern versus noncomplaint/nonconcern areas in the indoor environment.

Q: Does a correlation exist between culture-based and direct examination sampling?

A: There is no consistent correlation between measurement results from culture-based and direct examination samples for mold. However, even though these types of results cannot be directly compared, investigators may choose to incorporate both types of air sampling in their projects, as well as incorporate molecular methods, which may be even more specific than culturable methods. The types of information obtained from the different sampling types may differ; therefore, the type of sample collection to be used is dependent on the question(s) that sampling is intended to address.

Q: What are the differences and limitations of using direct examination versus culture-based methods?

A: The collection of air samples and determination of airborne fungal spore concentrations cannot be used to relate airborne concentrations to adverse health effects. The use of direct examination air sample results must be limited to ascertaining whether the two environments from which samples have been collected are different regarding fungal spore presence.

In addition, spore trap sampling for direct examination and analysis cannot be used for an "I just want to see what is in the air" project. Rather, such sampling can be used only to confirm or refute a hypothesis made by the IEQ professional based on an informed physical examination, such as, "The fungal spore presence in area A is not different from the fungal spore presence in area B."

Chapter 5 of AIHA's "Field Guide for the Determination of Biological Contaminants in Environmental Samples" (2nd edition) is a useful reference when considering spore trap sampling for direct examination as part of an indoor environmental investigation. Along with spores and hyphal fragments, nonbiological particles (e.g., soot and gypsum board dust) can affect the collection surface and can hinder the detection of fungal particles.

Although spore trap sampling for direct examination is not recommended in investigations that involve infectious fungal agents (because fungal culture/species identification would be required), such sampling and analysis can be used in evaluating levels of specific airborne allergens (e.g., fungal spores, fungal hyphae, pollen grains).

Direct examination results cannot be used for reliable species identification (often mistakenly referred to as "speciation"). Often, conclusively identifying a spore to the genus level is not even possible. For example, *Aspergillus* spores cannot be reliably distinguished from Penicillium spores in direct examination samples. This can be problematic when trying to discern whether two environments are the same or are not.

Environmental labs may differ in the fungal air sampling and analytical methods they recommend. The IEQ professional should confirm that the laboratory has experience in fungal analysis for the type of air sample collected.

Sampling and analytical error or uncertainty for spore trap samples is generally thought to be between 30 percent and 200 percent. Ideal samples with moderate spore loadings will have a sampling and analytical error closer to 30 percent, while samples with very high or very low concentrations of spores may have a sampling and analytical error closer to 200 percent. This analytical variability must be considered when comparing data from different samples.

It is widely known and reported by cognizant authorities that intra-day and spatial variations occur in airborne fungal spore concentrations. A typical spore trap sample is collected for five to 15 minutes. Spatial variations may be controlled if several samples are collected from several areas in a building over several time periods. Also, spore trap sampling for direct examination should not be used for environments with freezing temperatures.

Culture-based methods are limited by the length of time that is required to grow the sample in a laboratory (seven to 14 days). This time frame is often an issue with so-called "clearance" samples for which reoccupancy of a space is critical or preferred. However, it also can affect the length of time from the sampling to the onset of remediation. In addition, culture-based sample analysis can be more expensive than direct examination sample analysis.

Finally, owing to differences in growth factors among different kinds of molds, the sampling media that is chosen for the culture-based samples may not be appropriate to collect the airborne mold spores that are present in the space. As a result, spores of some genus and species may be collected, but these mold spores may not grow in the selected media. This factor should not only be considered in the IEQ professional's selection of the sampling media but also play a role in the IEQ professional's critical review of the report of analysis.

Q: What variables in environmental conditions can influence sample collection?

A: Variability in direct measurement is influenced both by the conditions of the sampling environment and by laboratory analyst-to-analyst variation. For the environment under evaluation, these considerations include conditions indoors and outdoors prior to and during the sampling. Some of these considerations are identified below:

- Type, operation, cleanliness and maintenance of the heating, ventilation and air conditioning (HVAC) system
- HVAC outdoor air supply rate and building air exchange rate
- Outdoor conditions, including season, weather, wind speed and wind direction
- General cleanliness of the indoor space
- Building envelope condition, such as windows or doors being open or closed
- Type, density and activity of occupants
- Processes and occupant use
- Activity near the sampler prior to and during sample collection

The variability imparted by these environmental conditions adds to the method, analytical, and temporal variability of sampling that needs to be considered when evaluating and interpreting any air sample results. The greater the variability of the environmental conditions, the more difficult it becomes to evaluate and interpret the results in relation to a hypothesis.

Q: What considerations should be given to temporal variabilities in airborne mold concentrations when sampling for mold?

A: Collecting a limited number of samples during a limited time period may not reflect actual airborne concentrations that occur over time. Research using multiple air samples collected periodically throughout the day in a room has shown that airborne concentrations collected in the same room at different times of the day can have a variability of as much as a 10,000-fold difference in detected concentrations. Day-to-day variations also occur, indicating that airborne fungal levels are episodic and are influenced by multiple environmental factors. Therefore, caution should be exercised when collecting and interpreting such samples.

Additional discussions on the concerns associated with temporal variabilities can be found in Chapter 5 of AIHA's "Field Guide for the Determination of Biological Contaminants in Environmental Samples" (2nd edition) and Chapter 10 of AIHA's "Recognition, Evaluation, and Control of Indoor Mold."

Q: When collecting and analyzing samples, what considerations should be given to account for analytical variability of results?

A: Inter- and intra-laboratory variations occur not only in fungal spore counts but also in the identification of fungal taxa. All accredited laboratories are required to maintain the laboratory's coefficient of variation (a measure of this

variability) for the method. This coefficient of variation must be considered when interpreting data. For example, the higher the coefficient, the greater the variability in the data and the greater the need for caution in interpreting and using the data.

Q: How many samples should be collected?

A: There is no single answer to the question of how many samples should be collected. The answer depends on the use and purpose of the sampling. In many cases, indoor bioaerosol sampling need not be performed at all if visible growth is observed during an informed mold inspection, because additional sampling information is not necessary to conclude that visible mold growth is present and needs to be addressed. Collecting and analyzing air samples does not and cannot evaluate health risks.

Before collecting any air samples for mold, the IEQ professional must determine the purpose of the sampling and identify the questions that the sampling results may answer. Air sampling should be considered only as a screening tool or as an ancillary tool for an informed inspection. In the absence of an informed inspection, air sampling alone cannot support definitive conclusions.

In addition, to address the question of the number of samples needed for a defensible sampling program, one must first recognize that bioaerosols, including molds, are normally found in air, both indoors and outdoors, and the types and prevalence can vary widely in time and space. Airborne mold spore counts are influenced by environmental and temporal factors, as described in the previous answers. The IEQ professional, therefore, should understand that, if it is determined that air samples are to be collected to evaluate a potential IEQ condition, multiple samples and repeat sampling over various time frames and modes of building operation and occupancy, along with a thorough visual inspection, including notation and consideration of local conditions that may affect sampling results, may be necessary to differentiate indoor and outdoor environments.

When sampling is to be carried out, the IEQ professional should determine whether the samples should be collected before, during or after the area is occupied, and whether the HVAC system is operating. At a minimum, the IEQ professional should understand that a single sample cannot be considered representative of an area. Because sample results can vary substantially (even for samples collected at the same time at the same location), the IEQ professional should evaluate multiple contemporaneous samples collected at each indoor location and each outdoor location at air intakes or other likely air entry points. At a minimum, a well-designed and well-executed sampling protocol that supplements an informed inspection is the only way that meaningful data from mold sampling in indoor environments can be evaluated.

Q: Does an analytical method to analyze airborne direct examination mold samples exist?

A: ASTM D7391-17e1 is a published, validated consensus method for laboratory analysis of air samples collected for the evaluation of total fungal airborne particles. This analytical method includes a discussion regarding bias and precision data, which must be documented for AIHA Laboratory Accreditation Program (AIHA-LAP)accredited direct examination laboratories. IEQ professionals submitting air samples should query the laboratory about what method of analysis it uses and whether the laboratory participates in proficiency testing and/or laboratory accreditation, to ensure that reported results meet minimum quality assurance/ quality control performance standards. It is strongly recommended that analysis be performed by labs that are accredited to ISO/IEC 17025:2017, General Requirements for the Competence of Testing and Calibration Laboratories.

Q: Is there an upper limit to spore counting results?

A: Theoretically, there is an upper limit for the counting of spores on a filter. If one were to place spores 3 micrometers (µm) in size perfectly side by side and in perfect columns (which is essentially impossible), then theoretically, one

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could fit 1 to 2 million spores on a slide. If the sampled air volume was 75 liters and the spores were perfectly aligned, the maximum spore count (using the assumptions above) would be millions of mold spores per cubic meter. Again, this assumes that the spores are the smallest of the commonly seen fungal spores and that they are placed perfectly side by side and in perfect columns.

On a practical basis, the laws of entropy limit the number of individual mold spores that can be counted on a slide, and that number is expected to be well below the theoretical maximum spore count. From a practical upper limit of quantification point of view, the overloading of sample media with debris, the overlapping of mold spores on the media, and the amount of time a laboratory is willing to spend on a sample heavily loaded with fungal spores are important considerations that will have a profound effect on the practical upper limit of quantification. Therefore, the IEQ professional should interpret with caution any laboratory results that report airborne mold spore counts greater than 1 million spores/m^3 of air.

When compared with asbestos loading of a similar nature, the NIOSH 7400 Method requires the lab to report such samples as having "greater than optimal variability" and as being "probably biased." Therefore, it is strongly recommended that the IEQ professional discuss these types of results with the lab directly to determine the proper interpretation.

Q: What is the difference between EMPAT participation and EMLAP accreditation?

A: There are several differences between a laboratory that is an AIHA Environmental Microbiology Proficiency Analytical Testing (EMPAT) participant and a laboratory that is accredited under the AIHA-LAP Environmental Microbiology Laboratory Accreditation Program (EMLAP).

Briefly, the only requirement to be an EMPAT participant is to pay the AIHA Proficiency Analytical Testing (PAT) annual fee and then purchase one or more of the proficiency tests. There are no other requirements to be an EMPAT participant. An EMPAT participant is not required to maintain proficiency to be listed as a current participant. Therefore, it is recommended that the investigator request a current EMPAT report from the laboratory to evaluate performance.

It is also important to understand that the EMPAT direct examination proficiency test evaluates only whether an analyst can identify the fungal taxa (genus, genus/species or group with commonality, like *Penicillium/Aspergillus*) on a digital image. No "real-world" samples are provided, and there is no requirement to perform quantification of mold spore concentrations (i.e., an airborne count).

To be EMLAP accredited, the laboratory must demonstrate proficiency through participation in EMPAT as well. However, accredited laboratories must also have a quality system that does the following:

- Rigorously evaluates each analyst's ability to quantify samples
- Continually monitors each analyst's performance
- Documents analyst training
- Documents analytical results
- Documents each analyst's precision and accuracy, if possible

EMLAP accredited laboratories must also do the following:

- Have in place corrective actions for nonconformance
- Perform duplicate and replicate analyses of client samples
- Participate in inter-laboratory sample exchanges
- Perform inter- and intra-laboratory statistical evaluations of data
- Be assessed on site every two years by an AIHA-LAP site assessor

These requirements enable AIHA-LAP to ensure that all the

required policies and procedures are followed and that the laboratory is performing at a level sufficient to receive the AIHA-LAP accreditation.

	EMPAT Participant	EMLAP Accredited Laboratory
Pays an annual fee to AIHA PAT programs	✓	✓
Purchases proficiency tests that identify taxa, not spore counts	✓	✓
Submits one composite result for proficiency analysis	✓	
All analysts must pass proficiency tests that identify species		✓
Documents annual training for analysts		✓
Continuously monitors analysts' performance		✓
Documents analysts' precision		✓
Performs and evaluates duplicate and replicate analyses of samples		✓
Participates in inter-laboratory sample exchanges		✓
Utilizes published or validated methodology		✓
Establishes and maintains a Quality Management System that is compliant with ISO/IEC 17025:2017		✓
Addresses nonconformities and implements corrective actions		✓
Has a documented continual improvement process		✓
Successfully completes an AIHA-LAP site assessment every two years		✓

The bottom line is that EMPAT participation consists only of participating in a proficiency testing program that identifies taxa. It is not equivalent to having an overall quality assurance (QA) program and process. To ensure that a laboratory's results meet all quality assurance requirements, IEQ professionals should be sure that the laboratory also has an ongoing QA program to evaluate quantitative results, analyst qualifications and management systems requirements. This is verified through the lab's participation in laboratory accreditation but can also be achieved with internal processes. Those using labs that are EMPAT participants only should also ask the lab for proficiency test results and quality assurance data (e.g., the lab's precision data).

For AIHA-LAP EMLAP accredited labs, IEQ professionals should ensure that the laboratory's scope of accreditation includes fungal air direct exam or fungal culturable evaluation, depending on the type of sample the IEQ professional intends to submit. Laboratory accreditation scopes can include other sample types, such as surface or bulk, so one should be sure to check that as well.

Note: Impaction air samplers and air sampling cassettes mentioned in this document are commonly used in the industrial hygiene/IEQ field. No endorsement is being made for any brand of air sampler or cassette. More information regarding air sampling instruments and devices can be found in Chapter 8 of AIHA's "Field Guide for the Determination of Biological Contaminants in Environmental Samples" (2nd edition) and Chapter 11 of AIHA's "Recognition, Evaluation, and Control of Indoor Mold," also known as the "AIHA Green Book."

To learn more, additional information can be found in the following resources:

American Industrial Hygiene Association: Facts about mold. Available at www.aiha.org/publications-and-resources/TopicsofInterest/Hazards/Pages/Facts-About-Mold.aspx.

Dotson, K.B., L.E. Patton, T.J. Ryan, J.V. Throckmorton, and D.M. Weekes: *Assessment, Remediation, and Post-Remediation Verification of Mold in Buildings*. Fairfax, Va.: AIHA, 2004.

Hung, L.L., J.D. Miller, and K.H. Dillon: *Field Guide for the Determination of Biological Contaminants in Environmental Samples*, 2nd ed. Fairfax, Va.: AIHA, 2005.

LeBouf, R., L. Yesse, and A. Rossner: Seasonal and diurnal variability in airborne mold from an indoor residential environment in northern New York. *J. Air & Waste Manage Assoc. 58*:684–692 (2008). https://doi.org/10.3155/1047-3289.58.5.684.

Macher, J.: *Bioaerosols: Assessment and Control*, 2nd ed. Cincinnati, Ohio: ACGIH, 1999.

Miller, J.D.: Fungi and the Building Engineer. Presented at IAQ 92: Environments for People, San Francisco, Calif., Oct. 19-21, 1992.

Prezant, B., D.M. Weekes, and J.D. Miller: *Recognition, Evaluation, and Control of Indoor Mold*. Fairfax, Va.: AIHA, 2008.

Spicer, R.C., and H.J. Gangloff: Bioaerosol data distribution: probability and implications for sampling in evaluating problematic buildings. *Appl Occup Environ Hyg. 18(8)*:584-590 (2003).

Spicer, R.C., and H.J. Gangloff: Verifying interpretive criteria for bioaerosol data using (bootstrap) Monte Carlo techniques. *J Occup Environ Hyg. 5(2)*:85-93 (2008).

Spicer, R.C., and H.J. Gangloff: Permutation/randomization-based inference for environmental data. *Environ Monit Assess. 188*:147-159 (2016).