Testing for airborne fungal spores: How to use the results derived from culture method?

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Introduction

Sampling and testing for airborne fungal spores is a practice that has been very popular with IAQ professionals and practitioners during a mold assessment and investigation, a routine IAQ survey, or air monitoring and clearance sampling during mold remediation. Although the spore trapping and microscopic analysis approach has been very popular over the last 5-10 years, there is very important information that spore trapping and microscopic analysis can not provide, such as viability of the spores. Negative results from the spore trapping and microscopic analysis are often inconclusive. Furthermore, the spore trapping and microscopic analysis has very little scientific literature to support its validity and usefulness. Collecting airborne spores onto nutrient agar medium with equipment, such as an Andersen N6 single stage sampler, is a time-tested practice. The medium is then incubated at room temperature or other selected temperature, depending on one's purpose, to allow deposited spores to germinate and grow into colonies for counting and identification. The design and study of the Andersen sampler was first published in the scientific literature by a US Army microbiologist in 1958. Since then, there has been a wealth of scientific literature evaluating the sampler and its application in indoor air sampling.

There are no numerical standards or guidelines regarding results of air samples of fungal spores derived through microscopic counting or by culturing. It is not very likely that such standards and guidelines will be available in the near future. Airborne fungal spores change frequently according to spatial and temporal variations. Without numerical standards and guidelines, the current approach recommended by professional or trade organizations to the interpretation of the results relies on comparisons of indoor vs. outdoor results and complaint vs. non-complaint area results. However, more important information in the results can be gathered if a practitioner knows how to interpret the results effectively.

This technical information sheet discusses how to interpret results of air samples collected during a mold evaluation, investigation or remediation and analyzed by the culture method.

Why select culture analysis method?

Culture analysis results include proper identification of fungi and their colony counts. Fungal identification, or qualitative analysis, is much more useful than concentrations (quantitative analysis), because concentrations are variable and easily influenced by temporal and spatial variations as well as biological nature (reproduction and death of spores). The spores deposited on the agar medium germinate and develop into colonies. This implies that the spores are viable and capable of growing. Viable spores of opportunistically pathogenic fungi can germinate, grow and cause infections in susceptible individuals. Viable spores will germinate and grow when encountering excessive moisture or water, leading to mold growth. More importantly, biological and ecological information of properly identified fungi can be easily researched and obtained.

Such information is useful in the result interpretation, retrospective assessment, and eventually mold control.

What to consider when comparing results of air culture samples

Different air samplers may use different flow rates (e.g. Andersen at 28.3 lpm v. SAS at 90 or 180 lpm) and have different collection efficiency due to design differences. Different agar media (MEA, CMA, DG18, or others) used in sampling can also yield results with variations. Therefore, **never** compare results derived from different air sampling equipment (e.g., Andersen v. Biotest). In addition, different sampling times (duration) may also yield different results. There are currently several types of air sampling devises widely used in sampling and testing for culturable fungi. Andersen, SAS, Biotest, & Burkard are examples of such devices. Fungal structures (mostly spores) are allowed to impact on agar surfaces, germinate, and grow into colonies. Typically they have a seven-day incubation period. Culturable methods usually yield lower fungal concentrations than spore trap methods because many airborne spores may be non-viable, dormant, or unable to germinate and grow on the media used. But culturable methods give proper identification of fungal colonies.

Interpretation of Airborne Spore Results Derived from the Culture Method

A detailed process on result interpretation is discussed below in this technical information sheet. Please remember that background information, on-site observations, and history of the case and building (such as water damage history and humidity problems) are very important in the final interpretation and conclusion. Results from other samplings, such as bulk, wipe or dust, from the same environment are also important. For example, *Cladosporium* taxa were found growing on bulk samples taken in a bathroom. This fact must take into consideration when *Cladosporium* spores are found in indoor air samples. Don't count airborne *Cladosporium* spores out, thinking they are phylloplane fungi and from outdoors. The information is also important when the results are used for remediation quality assurance or clearance purposes. Make sure that you also incorporate and use the information that you collect during your field study and sampling. Make use of floor plans or blue prints to map out your data. This may allow you to correlate the results with locations better.

The interpretive process is divided into several steps. You may or may not use all steps. If you feel any step is not applicable in your case, you may skip and go on to the next step. If you have any questions in the process, we are a phone call or an e-mail away.

i. If you have a large database of air sample results derived from the culture method, you may be able to define what is considered low, moderate, or high to screen your results. The database should be derived from air samples collected with the same sampler, on the same nutrient media, and analyzed in the same manner (such as standard incubation temperature). However, use such data with great caution and for performance evaluation only. The data should not be used for health evaluation criteria. Also keep in mind that numbers in microbiology are relative. They are used for comparison.

ii. Compare total concentrations from indoors, outdoors, complaint, and non-complaint areas. In general, indoor concentrations should be lower than those of outdoors. However, this may not always be consistent. Residential buildings, warehouses, schools, buildings with many entrances, buildings with operable windows, and buildings with

HVAC systems having no effective filtration may have airborne fungal levels higher than or as high as those outdoors. Results of non-complaint areas should consistently be lower than those of complaint areas if mold growth is an issue.

iii. Compare fungal types and species, indoors v. outdoors and complaint v. non-complaint areas. Fungal types from indoors and outdoors and complaint and non-complaint areas should generally be similar qualitatively. However, in a large building, such as a convention center or a 30-story office building, indoor fungal taxa may not always reflect what are outdoors because of air dilution due to the large air spaces in these buildings. In an airtight and mechanically ventilated building, indoor fungal taxa may include a collection of outdoor fungi over several days.

iv. Compare the data set of complaint-area samples to determine what fungal taxa are consistently detected. Evaluate the entire data set of complaint area samples to determine whether the complaint area has a consistent presence of certain fungal taxa or not. For example, ten complaint area samples are collected and all samples have *Aspergillus versicolor* at low concentrations. This suggests that the fungus may be near or at the location.

v. Know your fungi. Look for marker or signature fungi. Some fungal taxa, if expertly identified and detected indoors, are very likely associated with water damage, condensation issues or high humidity control problems. They are: *Aspergillus sydowii, A. versicolor, Penicillium chrysogenum*, and species of *Chaetomium, Stachybotrys, Memnoniella,* and *Ulocladium,* which are moisture-loving fungi. Spores of the last four genera are very rare in a clean, dry environment. These are moisture-loving fungi. They are essentially always associated with wet conditions (usually long-term) or significant and chronic condensation problems. Any detection of the four indicator fungi, *Chaetomium, Stachybotrys, Memnoniella,* or *Ulocladium,* in an indoor sample is important. Further investigation (or clean up if it is in a remediation) is strongly recommended. *Eurotium* is a sexual state of several *Aspergillus* species and a genus composed of xerophilic fungi. Its presence indicates persistent high relative humidity, poor ventilation and condensation problems. *Trichoderma* species also like wet conditions and can be a good indicator of water damage. They like to grow in damp basements and on wet wood. There are many other unusual fungal taxa associated with water damaged environments. Research and know your fungi. Call or send us an e-mail if your reports have such fungi.

vi. Consider concentrations and frequency of detection of marker or indicator fungi together. For example, *Aspergillus versicolor* was detected at low concentrations varying from 10 to 20 CFU/m^3 (v. *Cladosporium* at 1200 CFU/m^3), in 15 out of 20 indoor samples. With detection in 75% of the indoor samples and knowing that *A. versicolor* is an important water-damage marker fungus, the results strongly indicate samples from a water-damaged environment.

vii. Consider seasonal effects of airborne fungi. Indoor fungal growth may become inactive during the winter heating season unless there are persistent leaks or water sources to sustain the growth. Therefore, low airborne fungal spore levels in winter do not necessarily suggest a "clean or healthy" environment.

viii. If spores of *Stachybotrys chartarum* (synonym *S. atra*) are detected and the condition suggests growth of the fungus, and most likely other fungi, consult the "Guidelines on Assessment and Remediation of Fungi in Indoor Environments" published by the New York City Department of Health and Mental Hygiene. This

document is available from http://www.nyc.gov/html/doh/html/epi/moldrpt1.shtml. A new, revised version was published in November 2008. See the New York City website.

xi. Relate and correlate complaints, field observations, and laboratory results to determine whether fungal contamination and growth occurs in the building, complaint area or not. Remember moisture and water are the critical factors in indoor fungal growth. If there is fungal growth, there must be moisture or a water problem nearby.

x. Understand the ecology and background of the fungal spores identified. Some fungi grow at high water activity conditions. Species of *Chaetomium, Memnoniel1a, Stachybotrys* and *Ulocladium* require high water activity. The detection of their spores suggests consistently wet conditions. Another group of fungi are xerophilic and grow at low water activity. Some common xerophilic fungi found indoors are *Aspergillus penicillioides, A. restrictus, Wallemia sebi,* and species of *Eurotium.*

For those who are interested in learning more about air samples by culture analysis and the results derived from it and the ecology of indoor fungi, please consult the references listed below.

References

Yang, C. S. and P. Heinsohn. 2007. Sampling and analysis of indoor microorganisms. John Wiley & Sons, Hoboken, New Jersey.

Yang, C. S. and De-wei Li. 2007. Ecology of fungi in the indoor environment. p. 191-214. in "Sampling and analysis of indoor microorganisms." John Wiley & Sons, Hoboken, New Jersey.