Fungal spores in the Air: How to use the results of spore count?

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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 monitoring and clearance sampling during mold remediation. There are no numeric standards or guidelines regarding results of air samples of fungal spores. It is not very likely that such standards and guidelines will be developed in the near future. Airborne fungal spores change frequently according to spatial and temporal variations. Without 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, the results can be much more useful to the practitioners if one knows how to interpret the results effectively.

This technical information sheet discusses how to interpret results of air samples analyzed for airborne fungal spores collected during a mold evaluation, investigation or remediation.

What to consider when comparing spore count results of air samples

Different air samplers and devices use different flow rates and have different collection efficiency. Therefore, **never** compare results derived from different air sampling equipment or devices. In addition, different sampling time (duration) may also yield different results. There are currently several spore-trapping equipment and devices widely used in sampling and testing for total airborne fungal spores. Spore traps use AllergencoD cassettes, Air-O-Cell cassettes, Allergenco sampler, or Burkard samplers to collect spores and dust onto a greased-coated receiver. All fungal structures (including spores, conidiophores, and hyphal fragments) are counted and **presumptively** identified. Interpretation of total spore count results is similar to the culturable results. Spore trap methods generally yield higher levels of fungal structures but identifications are presumptive at best. In post-remediation quality assurance (a.k.a. clearance) sampling, spore trap methods offer the advantages of detecting dead spores from biocide treatments and quick turnaround time. Culturable methods usually yield lower fungal concentrations than spore trap methods because many 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 Fungal Spore Results Derived from the Spore Counting Method

A detailed process on result interpretation is discussed 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* was found growing on bulk samples taken in a bathroom. This fact must take into consideration when *Cladosporium* spores are found in air samples. Don't count

airborne *Cladosporium* spores out thinking they are 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 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 question in the process, we are a phone call or an e-mail away.

i. If you have a large database of airborne fungal spores derived from total spore counting method, you may be able to define what is considered low, moderate, or high to screen your results. 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 that of outdoors. However, this may not be always consistent Residential buildings, warehouses, schools and buildings with many entrances and openable windows, and buildings with HVAC systems with no effective filtration may have airborne fungal levels higher than or as high as that of outdoors. Results of non-complaint areas should consistently be lower than that of complaint areas if mold growth is an issue.

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

iv. Compare the dataset of complaint-area samples with results from outdoors, non-complaint area, or both, to determine what are the fungal spore type(s) uniquely detected in complaint area(s). Evaluate the entire dataset of complaint area samples to determine whether the complaint area has consistent presence of certain fungal spores or not. For example, ten complaint area samples are collected and all samples have *Chaetomium* at low levels. This suggests that the fungus may be near or at the location.

v. Look for marker or signature fungal spores. Some fungal spores, if expertly identified and detected indoors, are very likely associated with water damage, condensation issues or high humidity control problems. They are: spores of *Chaetomium, Stachybotrys, Memnoniella, Ulocladium*, and *Eurotium*. Reproductive structures of these fungi, such as ascus, conidiophores, or ascomata, are also useful information to have since it indicates active growth. *Chaetomium, Stachybotrys, Memnoniel1a*, and *Ulocladium* are moisture-loving fungi. Spores of these four genera are very rare and unusual in a clean, dry environment. They are essentially always associated with water damage or with significant and chronic condensation problem. *Eurotium* is a sexual state of several *Aspergillus* species and a genus of xerophilic fungi. Its presence indicates persistent high relative humidity, poor ventilation and condensation problems, Keep in mind that spores and structures of these fungi may also come from outdoors, although the possibility is low. *Trichoderma* also likes wet conditions and can be a good indicator of

water damage. They like to grow with wet wood and are commonly in wet basements or crawlspaces. However, *Trichoderma* spores are not particularly characteristic and are often grouped with *Aspergillus/Penicillium*-like spores. You may consider *Trichoderma* spores as a marker of wet conditions, if you have confidence in the identifying laboratory. *Aspergillus/Penicillium*-like (or Pen/Asp-like) spores are often used by uninformed laboratories and practitioners as indicative of water-damaged conditions. They are very common indoors and outdoors. If *Aspergillus/Penicillium*-like spores of indoors are statistically, consistently, and significantly higher than that of outdoors, then the difference is significant for a water-damaged environment.

vi. Consider seasonal effects of airborne fungal spores. Indoor fungal growth may become dormant during 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.

vii. 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 is expected in second half of 2008.

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

ix. If results of more than one sample types are available, correlate the results. For example, *Cladosporium* is found growing on drywall samples collected indoors. In this case, indoor airborne *Cladosporium* spores are not exclusively from outdoors.

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 species of *Eurotium*.

xi. If indoor hyphal fragments are statistically significant higher than that of outdoor in post remediation sampling, this suggests additional cleaning is necessary.

xii. If through the comparative process the results between indoors and outdoors, or complaint and non-complaint areas are not significantly different, do not jump into the conclusion that the environment is fine. This is particularly true when the number of sample collected is limited and spore counting is the only analysis used. In this situation, the only conclusion is the results are inconclusive.

Ecology of common airborne spore types.

1. amerospores. Some laboratories of questionable qualifications often identify this group of spores as small, rounded spores. The mycological definition of amerospores is asexual spores that are "non-septate spores with a length/width ratio not exceeding 15: 1 ", This definition includes a wide variety of spores, including *Aspergillus/Penicillium*-like,

2. ascospores and asci: Ascospores are sexual spores produced in an ascus or asci (sacs) by ascomycetes (including cup fungi, *Chaetomium* spp., *Eurotium* spp., and *Peziza*

spp.) Their morphology is very variable and diverse. They are abundant during warm months of the year.

3. basidiospores: Basidiopsores are sexual spores produced on a basidium or basidia by basidiomycetes (including mushrooms and toadstools, bracket and cone fungi, jelly fungi, rusts and smuts). Most basidiospores have similar characteristics but variations are common. They are in abundance in spring and in late summer to fall after a significant rainfall or a period of wet days. Their source is mostly outdoors. Indoor source may be from wood decay fungi due to long-term moist, wet conditions.

4. conidiophores: Conidiophores are specialized hyphae on which asexual spores (conidia) are produced. Conidiophores are very important in the identification and speciation of microfungi, which are very common indoors and include *Aspergillus, Penicillium, Memnoniella, Stachybotrys*, and others. Some conidiophores are very characteristics and identifications can be made based on conidiophores, Their presence can be a good indication of fungal growth indoors. They are very rare in outdoor air.

5. hyphal fragments: Hypha (pl, hyphae) is a vegetative, filamentous fungal structure from the growth. It presence possibly signals presence and growth of fungi. It is unusual for hyphae or hyphal fragments to become airbome unless it is disturbed, such as in a mold remediation. Elevated levels of hyphal fragments indoors may suggest indoor growth and disturbance.

6. myxomycetes. Myxomycetes are commonly called slime molds. Mycologists consider them not to be true fungi, but mycologists study them. Spores of myxomycetes are variable and their sources are mostly outdoors. Some fruiting bodies of myxomycetes have occasionally been identified from indoor samples. Without fruiting bodies, spores of myxomycetes are considered of outdoor origin. Their growth often occurs in hot, humid summer.

7. rusts. Rust spores are produced by a group of obligate parasites in the basidiomycetes. They infect trees and a wide variety of plants. They are called rusts because their spores in mass are in rust color. Their sources are always outdoors.

8. smuts: Smut spores are produced by a group of parasitic basidiomycetes. They infect grasses and produce most black (other colors are possible) spore mass in mid- to late growing season (such as summer). The well-known corn smut is a species associated with corn and is edible. Their source are always outdoors.

9. zygomycetes. Zygomycetes produce zygotes in their sexual reproduction, which is occasionally observed. Several species of Zygomycetes are well known and may grow indoors. A few examples of common zygomycetes are *Absidia corymbifera, Mucor pleumbus, Rhizopus stolonifer,* and *Syncephalastrum racemosum*. Their spores are very variable. Some are very simple and some are very characteristic. Laboratory reports, which list zygomycete spores, are often of questionable quality.

10. *Altemaria*: *Alternaria* spores are relatively characteristic due to its size, shape, and septation. However, several other fungal species and genera (such as *Mystrospriella, Phoma glomerata, P. pomorum, Ulocladium*) produce spores that are very similar or almost identical to *Alternaria* spores. Poorly trained analysts and their laboratories can easily mis-identify the spores.

11. *Aspergillus/Penicillium*-like (or Pen/Asp-like): Spores of *Aspergillus* and *Penicillium* are in most cases very simple. Between them, there are approximately over four hundred species. They are mostly small, rounded or slightly off, and smooth to ornamented. There are many fungal genera and species which produce spores that are very similar or difficult to differentiate from *Aspergillus* and *Penicillium*. Spores of the

following groups may be identified as *Aspergillus/Penicillium*-like spores. They are. *Aspergillus*, *Aureobasidium, Penicillium, Trichoderma*, *Absidia*, Acremonium, *Aphanocladium, Beauveria, Chromelosporium, Cladosporium* (young spores*), Phialophora, Gliocladium, Metarrhizium, Monocillium, Mortierella, Mucor, Paecilomyces, Thysanophora, Torulomyces, Verticillium*, amerospores, ascospores, basidiospores, yeasts, etc. Please be forewarned. Us results of *Aspergillus/Penicillium*-like spores with great cautions.

12. *Aureobasidium*: Although *Aureobasidium* is a very common fungal group, their spores are not very characteristic. A few laboratories have reported spores of *Aureobasidium*. Their identification is highly questionable.

13. *Botrytis*: Spores of *Botrytis*, particularly *B. cinerea*, are common outdoors. *B. cinerea* grows in cool, damp weather with stagnant air and is common on plants and fruits with excessive accumulation of water. The spores are of outdoor origins, although they may be from growth on fruits or flowers brought in from indoors. Other species of *Botrytis* may be encountered but their ecology is similar.

14. *Cercospora*. Species of *Cercospora* are associated with a wide variety of plants. They are weak parasites on dead, dying, or, occasionally, on healthy plants. The spores are likely of outdoor sources. *Pseudocercospora*, and ascospores of *Balansio*, *Cochliobolus*, and *Gaeumannomyces*, and other genera produce similar spores.

15. *Chaetomium*: *Chaetomium* is an ascomycetous genus. *Chaetomium* species produce ascospores, which are very characteristic and easy to identify. A similar genus, *Chaetomidium*, produces similar spores. *Chaetomium* species grow on wood and paper products, and are hydrophilic (moisture-loving or high water activity). They are common on water-damaged drywall, wood, or materials with significant cellulose content. Spores of *Chaetomium* detected indoors are excellent indicators of water damage.

16. *Cladosporium*: Spores of *Cladosporium* are the most common airborne spore type on the earth. However, many other fungi (see below) also produce spores that are indistinguishable or very similar to *Cladosporium* spores. They are considered from outdoors. However, species of *Cladosporium* can grow indoors in the HVAC in association with fibrous insulation materials and on cold, condensing surfaces. Therefore, they are not exclusively outdoors if *Cladosporium* growth is detected indoors. Spores of *Cladophialophora, Exophiala, Fulvia, Gonatobotryum, Hormoconis (Amorphotheca), Hyalodendron, Mycovellosiella, Periconiella, Phaeoramularia, Septonema, Stenella* are indistinguishable or very similar to *Cladosporium* spores.

17. *Curvularia*: Spores of *Curvularia* are very characteristic and easy to identify. They grow on a wide variety of plants. The source of the spores is most likely outdoors.

18. *Drechslera/Bipolaris*: Spores of *Drechslera/Bipolaris* are characteristic and readily identifiable. They grow on a wide variety of plants. The source of the spores is most likely outdoors.

19. *Epicoccum*: Spores of *Epicoccum* are common outdoors. However, *Epicoccum nigrum* can grow on water-damaged building materials.

20. *Eurotium*: *Eurotium* species produce characteristic ascospores and recognizable to experienced analysts. *Eurotium* species are highly xerophilic and grow under extended high humidity conditions, such as wood in crawl space.

21. *Fusarium*: *Fusarium* may produce more than one type of spores. Some species consistently produce both macro-conidia and micro-conidia. The macro-conidia are sickle-shaped with tapered ends and easily identified. The micro-conidia are, however, difficult to identify. *Cylindrocarpon, Acremonium, Gliocladium, Microdontium*, and

Monographella may produce spores similar to *Fusarium* spores. Spores of *Fusarium* are generally considered of outdoor origins. However, a few species of *Fusarium* can grow indoors in chronically wet or damp areas (such as drain pans of HVAC).

22. *Ganoderma*: *Ganoderma* is a basidiomycetous genus. They produce bracket or conk mushrooms on trees. Their basidiospores are very common in outdoor air during season.

23. *Memnoniella*: The genus *Memnoniella* is differentiated from *Stachybotrys* by their different spore shapes and whether spores are in chains (*Memnoniella*) or in slimy mass (*Stachybotrys*). However, recent studies and publication by us indicate that *Memnoniella* may produce *Stachybotrys*-like spores. *Memnoniella* spores can be identified by properly trained laboratory analysts. They are excellent indicators of water-damaged environments.

24. *Nigrospora:* Spores of *Nigrospora* are very characteristics. Species of *Nigrospora* grow on a variety of plants. They are known to rarely grow on water-damaged materials.

25. *Pithomyces*: Spores of *Pithomyces* are cosmopolitan and very characteristics and easy to identify. However, poorly trained laboratory analysts may mis-identify and reports from many laboratories show that spores of *Pithomyces* may be confused with spores of *Ulocladium*. *Pithomyces* species grow with plants and have not been reported or observed to grow indoors. *Ulocladium* on the other hand is an excellent indicator of water-damaged environment (see below).

26. *Rhizopus*: This zygomycetous genus produces characteristic spores that are easily distinguishable by trained laboratory analysts.

27. *Scopulariopsis*: Spores of this genus are very characteristic and easily distinguishable by trained laboratory analysts. Several species of this genus grow on water-damaged wallpapers and in dusts of damp environments. They are excellent indicators of water-damaged environments. *Doratomyces* and *Trichurus* produce similar spores.

28. *Spegazzinia*: Spores of *Spegazzinia* are very characteristics and easy to identify. Their sources are of outdoor origins, including plants and soils.

29. *Stachybotrys*: Spores of *Stachybotrys* are very characteristics and can be confidently identified by properly trained laboratory analysts. *Stachybotrys* species grow on paper products, and are hydrophilic (moisture-loving or high water activity). They are common on water-damaged drywall or materials with significant cellulose content. Spores of *Stachybotrys* detected indoors are excellent indicators of water damage. Spores of *Memnoniella, Gliomastix, Periconia* may be mis-identified as spores of *Stachybotrys*.

30. *Stemphylium*: Spores of *Stemphylium* are large and characteristic, although confusion and mis-identification with *Monodictys* is likely. Some species of *Monodictys* grow on wood and are common on water-damaged wood indoors. *Stemphylium* grows mostly with herbaceous plants and occasionally with wood. Because of their sizes, they are not very common in the air.

31. *Torula herbarum*: Spores of this species is very characteristic for identification. Their sources are most likely outdoors, such as herbaceous plants, soil, wood, etc.

32. *Ulocladium*: Spores of *Ulocladium* are characteristic and can be identified with confidence by properly trained laboratory analysts. Spores of *Alternaria*, *Monodictys,* or *Pithomyces* may be confused with *Ulocladium*. There have been

laboratory reports grouping spores of *Pithomyces* and *Ulocladium* together. *Ulocladium* spores, if properly identified, are excellent indicator of water damage.

Protocol for Collecting Airborne Spore Trap Samples for Total Spore Count

1. If you use sampling equipment, such as Burkard or Allergenco samplers, follow the manufacturer's suggestions. These samplers usually collect for one to ten minutes at a set flow rate. However, under unusual conditions (such as a clean room or a hospital isolation area), longer sampling time may be used.

2. If you prefer using spore trap devices, such as Air-O-Cell cassettes or others, follow the manufacturer's suggestions. We recommend use those that have been scientifically evaluated and published in scientific literature. These samplers usually collect for five to ten minutes at a set flow rate (usually 15 lpm).

3. Disinfect or clean the sampler with 70% rubbing alcohol, and allow alcohol to dry before loading your grease-coated slides.

4. Follow the manufacturer's instructions to take your samples. Label and seal your sample cassettes, or place your slide samples in a clean slide box.

5. Complete your C-O-C sheet. Keep a copy for your record.

6. When taking spore trap samples, total air volume between 50-150 L is suggested. Dirty and dusty air, such as in a remediation containment area or in a composting facility, lower air volume (15-75 L) is recommended.

7. Place your grease-coated slide in a slide box. If the slide box is re-useable, wash it to avoid contamination. Cassette samples should be securely sealed and taped for shipping. Do not ship your samples in an envelope, whether padded or not. Ship your samples in a box. Have them delivered to our laboratory as soon as possible if you would like to have a quick turnaround for the results.

For those who are interested in learning more about spore counting and results derived from it and the ecology of such 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.

Li, De-wei, C. S. Yang, and F. Harrington. 2007. Microscopic analytical methods for fungi. p. 75-103. in “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.