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The Collection and Interpretation of Wall Cavity Samples

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There seems to be a significance divergence in views among mold investigators concerning the utility of sampling wall cavities for mold. Some think it is an indispensable tool when performing mold investigations, while other investigators will argue that this technique has little merit. So, is it even worthwhile to collect wall cavity samples? I'm one of those investigators who say "yes." Allow me to explain why by addressing the following questions:

– Why sample wall cavities?
– Which wall cavities should be sampled?
– What about the sample volume?
– What are we looking for in wall cavities?
– How should wall cavities be sampled?

Why sample wall cavities?

There are five basic reasons that I collect wall cavity samples. The five reasons are:

– Eighty percent of the houses I am asked to investigate do not have any visible mold;
– To find old leaks, using mold as a surrogate for moisture;
– To identify sites for destructive testing, and confirm the results of the destructive testing;
– To define a scope of remediation, or assess the effectiveness of a mold restoration; and
– To assess whether or not hidden fungal reservoirs are affecting the indoor environment.

Reason #1

The primary reason I use wall cavity sampling is that I am more likely to encounter hidden fungal reservoirs than visible mold growth during an investigation. Probably more than four out of five houses I am asked to investigate do not have any visible mold, whereas probably two out of three houses have hidden mold. Yet, even though the mold is hidden, the client was still concerned enough to ask for an investigation. Many water intrusions that result in mold growth initially involve wall cavities and other inaccessible areas of the structure. The mold remains hidden from view, and it only becomes visible if the water intrusion was so intense that mold actually grew through the drywall or other material.

Reason #2

The second reason I use wall cavity sampling is to locate areas of current, as well as past, water intrusion. Although I am sampling for mold, mold follows moisture. Therefore, mold can be used as a surrogate for moisture. For example, suppose a home owner states that during the last rain, a significant leak occurred in the living room window. However, if the last rain was six months ago, the wall will be dry, and a moisture meter is not going to be very useful in verifying that the leak occurred. However, if significant amounts of water did enter the wall cavity during the last rain, there is a good chance that detectable amounts of mold spores will still be present in the wall cavity under the window.

The sampling device that I use (Bi-Air cassette) collects both fungal spores and culturable fungi in the same sample. Collecting both spores and fungi offers several advantages, and one of those advantages is that the results can be used to somewhat "date" the water intrusion. The data in Table 1 illustrate this concept.

As a wet wall cavity becomes dryer, the percentage of spores that remain viable (alive) tends to decrease. The mold spores begin to die as the wall dries. If the percentage of culturable fungi are used to estimate the percentage of viable fungi in the wall cavity, then we see that a much higher percentage of spores were still alive in Wall # 1 as compared to Wall # 2. Although this is not a very precise timing method, it may provide some insight into which walls were damaged by the current water intrusion (Wall # 1) as opposed to some previous water intrusion (Wall # 2).

Reason #3

The third reason I collect wall cavity samples is to identify the most productive sites for destructive testing, as well as to confirm the results of the destructive testing. Destructive testing is relatively expensive, it disrupts the occupants living environment, and it may be subject to false negatives. Therefore, in many mold investigations, it is used sparingly.

I recently performed a mold investigation on a house that had 118 windows, some of which leaked. Using wall cavity sampling, it was possible to identify a group of windows for destructive testing. This was accomplished at a relatively small cost, and minimized both the disruption and cost of the destructive testing by "pre-qualifying" the test sites.

The statement that "fungal spores and hyphae are invisible to the unaided eye" is readily accepted by most mold investigators. But, the corollary "therefore visual inspection of surfaces for fungal contaminants, by itself, is inadequate to detect the presence of fungal contaminants" seems to be highly controversial. Figure 1 contains a photograph of the interior surfaces of five pieces of drywall. The five surfaces were judged to be "clean" by three separate individuals at the time the samples were collected.

As indicated in Table 2, two of the pieces of drywall had a surface growth of culturable *Aspergillus* and/or *Penicillium*, and came from wall cavities with significant concentrations of culturable *Aspergillus* and *Penicillium*. In this particular instance, visual inspection was not an acceptable method for detecting the presence of significant concentrations of fungal contaminants that were present inside the wall cavity.

I sometimes hear about wall cavities that were sampled and found to contain mold, but were "perfectly clean" when the wall was opened during destructive testing. This is often used as an argument that wall cavity sampling is prone to "false positives" (laboratory reporting spores when they were not really there). Personally, I find it difficult to grasp the logic of such an explanation (a spore that was not there grows and produces a fungal colony in a lab culture?). It is more probable that spores and hyphae were present, but that they were not detected by visual inspection when the wall was opened.

Reason #4

The fourth reason for sampling wall cavities is to assist the occupants in fulfilling their objectives. I may be asked to define a scope of remediation, help them obtain legal assistance, or assess the effectiveness of a recently completed mold restoration (as opposed to a mold remediation). It is my experience that wall cavity samples are generally required to adequately accomplish each of these tasks. For example, it

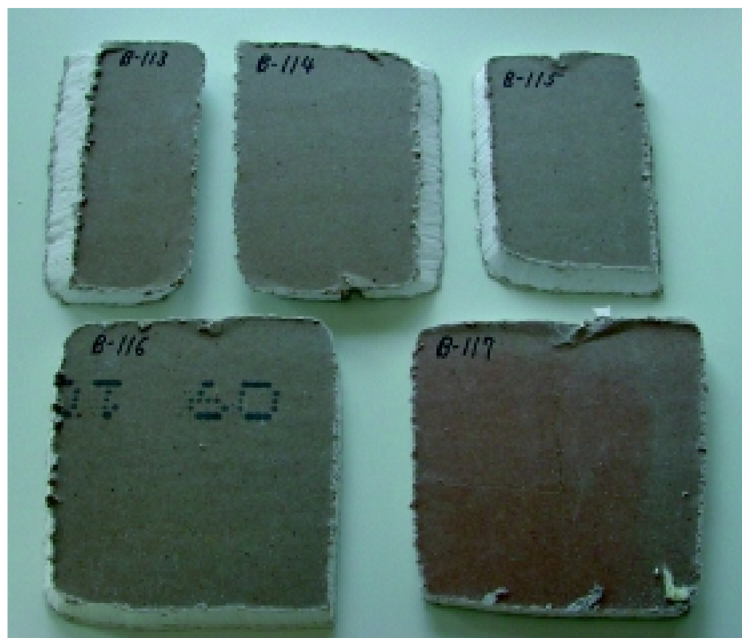

Figure 1
Interior surfaces of five pieces of drywall: two contaminated and three uncontaminated.

Table 1
Example Wall Cavity Data (Spores or Colony Forming Units Per Cubic Meter of Air)

Fungal Type	Wall #1	Wall #2
Aspergillus/Penicillium spores	100,000 spores/m^3	100,000 spores/m^3
Culturable *Penicillium*	90,000 cfu/m^3	10,000 cfu/m^3
Percent Culturable	90%	10%
WATER INTRUSION	Recent?	Previous?

Table 2
Wall Cavity Concentrations Associated with Visibly "Clean" Drywall Samples

Sample Number	Concentration (cfu/m^3)	Swab Samples
B-113	3,500,000	*A. versicolor*
B-114	370,000	*A. versicolor, Penicillium*
B-116	13,000	Sterile Fungi
B-115	less than 2,500	No Fungi Detected
B-117	less than 2,500	No Fungi Detected

is not unusual to sample directly in the drying holes cut in walls and toe kicks while drying the materials in place, and to then detect significant amounts of mold spores inside those cavities. Unfortunately, those drying holes provide an open pathway between the fungal reservoirs and the breathing zones of the occupants.

Reason #5

The fifth reason to collect wall cavity samples is to assess whether or not hidden fungal reservoirs are affecting the indoor environment. One study has concluded that fungi contained in intact wall cavities sometimes enter the indoor air [Morey P, Andrew M, Ligman B, Jarvis J. Hidden Mold Sometimes Enters the Indoor Air. In Indoor Air 2002: Proceedings of the 9th International Conference on Indoor Air Quality and Climate, Vol. 2, Levin H, ed., Indoor Air 2002, Santa Cruz, California, 2002, pp. 455-460].

In addition, it is not infrequent that even low concentrations of airborne *Aspergillus versicolor*, when detected persistently in air samples, indicate that hidden mold reservoirs are present in wall cavities. However, in order to detect such as association, both the air samples and the wall cavity samples must be cultured, and the fungi identified to the species level. This is the second advantage of using the Bi-Air cassette, which can collect both fungal spores and culturable fungi as part of the same sample.

Which wall cavities should be sampled?

Although wall cavity sampling is an essential part of my consultant's "toolbox," the indiscriminate sampling of wall cavities may not be very productive.

I generally confine wall cavity sampling to the following situations:

1. Plumbing walls: behind showers, toilets, sinks, laundries, wet bars.
2. Base cabinets: the toe kick area under base cabinets that have been wet.
3. Penetrations in perimeter walls: windows, sky lights, sliding glass doors, cantilevered beams.
4. Planter boxes: adjoining perimeter walls.
5. Exterior wall: large cracks, areas lacking drainage (covered weep screed, etc.), non-porous wall covering, sub-grade walls.
6. Irrigation problems: visible problems, high soil, poor drainage, etc.
7. Restoration: areas of inadequate remediation.

This is not an exhaustive list of potential sampling locations, but it does indicate the types of locations in which wall cavity sampling may prove useful.

What about the sample volume?

Let's calculate the volume of air contained in typical stud bays (space between studs) in houses, because that affects how much air can be withdrawn during sampling. A stud bay 8 feet high and 16 inches wide contains an air volume of about 79 liters, while a stud bay located under a window set at 30 inches contains about 27 liters of air. However, the area under the window is exactly where we want to collect many of our samples, so we will assume there are 27 liters of air in the "sample container."

If we are interpreting the laboratory results qualitatively (just what is there, not how much is there), then the volume of air we withdraw from the stud bay does not matter. However, if we decide to interpret the laboratory results quantitatively (not only what is there, but how much is there), then the volume of air withdrawn from the stud bay (the sample container) becomes important. The reason is that as air is withdrawn from the wall cavity, a like volume of fresh air is drawn into the wall cavity, diluting the sample.

As a practical limit, in order to maintain the integrity of the sample, no more than about 10 percent of the available air should be withdrawn during sample collection. If the sample is being drawn from a single stud bay located under the typical window, and if numerical guidelines are to be applied to the sample results, then the maximum sample volume should be limited to about 3 liters.

Also, as previously stated, mold follows moisture. A wall cavity subjected to a floor-level water spill will have most of the wetness localized near the baseboard. Therefore, I generally collect the sample near the baseboard, not in the middle of the wall. If the water intrusion occurred at the ceiling, gravity might still cause most of the wetness in a wall cavity to be localized near the baseboard. However, in that case, I will sample both near the baseboard and near the ceiling.

What are we looking for in wall cavities?

In a previously published study describing 150 wall cavity samples [Spurgeon JC. A method for detecting fungal contaminants in wall cavities. *AIHA Journal (Fairfax, Va)*. 2003 Jan-Feb;64(1):40-7.], only *Aspergillus* and/or *Penicillium* species were detected in 69 percent of the wall cavities in which culturable fungi were detected. This result, which was supported by other studies referenced in the article, suggests that culturable *Aspergillus* and/or *Penicillium*, or *Aspergillus/Penicillium* type spores, are frequently the primary "indicators" of a contaminated wall cavity. Therefore, basing numerical guidelines on "total spore counts," which may include Myxomycetes, rusts, smuts, etc., as an indication of contamination may tend to bias the conclusion.

The data in Table 3, which are similar to two actual samples I was asked to compare, illustrates this concept. The total spore counts in samples W-1 and W-2 are the same. However, the total spore count in sample W-1 is almost entirely due to basidiospores, rusts and smuts, with very few *Aspergillus/Penicillium* type spores detected. In sample W-2, over 90 percent of the total spores were due to *Aspergillus/Penicillium* type spores and *Cladosporium*.

When assessing the condition of a wall cavity, I generally focus on the total concentration of "indicator genera," which commonly include *Aspergillus, Chaetomium, Stachybotrys, Ulocladium*, etc. Therefore, I would conclude from these data that location W-1 had not been subjected to water intrusion, while location W-2 had been subjected to water intrusion.

I report my sample results as "standardized" data, whereas a number of reports I have reviewed did not. For example, reporting a result as "100 spores" is not standardized, and does not provide a basis for comparing that result with any other result. Reporting a result as "100 spores per cubic meter of air" is standardized based on the sample volume, and that result can be compared to other similarly standardized sample results.

Anyone who collects samples should know that "if you do not know how you are going to interpret the data, then do not collect the sample." So, can wall cavity data be interpreted? In my opinion, the answer is yes, in at least two ways.

The first method is by determining the dominant types of mold spores detected in the wall cavity. For example, *Stachybotrys chartarum* requires wet conditions for growth, while *Aspergillus versicolor* prefers near-wet conditions. As conditions in the wall cavity move down the moisture scale, then *Chaetomium* or *Ulocladium* may become dominant. Finally, in wall cavities that have dried out, *Penicillium* and *Cladosporium* tend to be dominant.

The second method is to use numerical guidelines to interpret the sample results. However, even though I am about to discuss numerical guidelines, professional judgment should always be included in the decision making process.

Let's discuss the WallChek sampling device first. I was asked to analyze a significant number of wall cavity samples collected using the WallChek. Without going into a lot of detail, I found a "discontinuity in the lognormal distribution" at about 7,000 spores/m^3 for *Aspergillus/Penicillium* type spores. A similar discontinuity was not observed for total spores.

Based on those samples collected using the WallChek: (1) data interpretation should be based on *Aspergillus/ Penicillium* type spores and other "indicator genera" rather than total spores, and (2) wall cavities with *Aspergillus/ Penicillium* type spore concentrations less than 7,000 spores/m^3 (uncontaminated) seemed to be different from those with concentrations greater than 7,000 spores/m^3 (contaminated).

Now, let's discuss the Bi-Air cassette, which collects both fungal spores and culturable fungi. In addition, it has a higher collection efficiency compared to the WallChek device, so the reported concentrations will be higher than those obtained with the WallChek. Tables 4a and 4b contain the numerical decision criteria I generally use when interpreting wall cavity data collected using the Bi-Air cassette.

Table 3
Example Sample Results for Two Wall Cavities (spores/m^3)

Sample	Wall #1	Wall #2
TOTAL SPORES	15,000	15,000
Aspergillus/Penicillium	500	8,000
Basidiospores	2,000	500
Cladosporium	500	6,000
Rusts, Smuts, *Myxomycetes*	12,000	500

Table 4a
Numerical Guidelines for Culturable *Aspergillus/Penicillium* (cfu/m^3)

Culturable *Asp/Pen*	Contaminated	Remediation
< 10,000	No	No
10,000 - 50,000	Possible	Possible
≥ 50,000	Yes	Yes

Table 4b
Numerical Guidelines for *Aspergillus/Penicillium* Type Spores (spores/m^3)

Asp/Pen Spores	Contaminated	Remediation
< 25,000	No	No
25,000 - 100,000	Possible	Possible
≥ 100,000	Yes	Yes

How should wall cavities be sampled?

In my opinion, a wall cavity sampling device should have the following characteristics:

- Collects both total fungal spores and culturable fungi;
- Has a high collection efficiency for fungal spores, and collection efficiency does not vary with either fungal or debris concentration;
- A clean sample probe is used for each sample;
- Has a solid sample probe that will not "crimp" inside a wall cavity;
- Has a low airflow rate to avoid the collection of heavy debris loadings, combined with a low sample volume to minimize sampling bias.

The Bi-Air cassette (http://www.moldinspectionproducts.com), which is the sampling device that I use, has all of these characteristics.

continued on page 64

Glossary of Terms

The following terms are found throughout this issue of *COLUMNS-Mold*. The definitions are from the 1995 edition of *Merriam-Webster's Medical Dictionary* unless otherwise stated.

antibody: any of a large number of proteins produced normally after stimulation by an antigen and act specifically against the antigen in an immune response

antigen: a substance capable of stimulating an immune response

apical: of, relating to, or situated at an apex (a narrowed or pointed end of an anatomical structure)

aspergillosis: opportunistic infections caused by *Aspergillus* sp and inhaled as mold conidia, leading to hyphal growth and invasion of blood vessels, hemorrhagic necrosis, infarction, and potential dissemination to other sites in susceptible patients. [http://www.merck.com/pubs/mmanual/section13/chapter158/158i.htm]

asthma: a condition often of allergic origin that is marked by continuous or paroxysmal labored breathing accompanied by wheezing, by a sense of constriction in the chest and often by attacks of coughing or gasping

biomarker: a distinctive usu. biochemical indicator (as a metabolite) of a biological or geochemical process or event (as aging, poisoning, fossilization, or oil formation) [http://www.m-w.com]

coenocyte: a multinucleate mass of protoplasm resulting from repeated nuclear division unaccompanied by cell fission [http://www.m-w.com]

conidia: generic term refering to a spore produced in vegetative reproduction of a fungus and located on sporulating blotches [http://www.inra.fr/Internet/Produits/HYP3/pgloss/6---120.htm]

control: one that controls: as 1. an experiment in which the subjects are treated as in a parallel experiment except for omission of the procedure or agent under test and which is used as a standard of comparison in judging experimental effects — called also control experiment 2. one (as an organism, culture, or group) that is part of a control [http://www.m-w.com]

cytokine: any of a class of immunoregulatory substances (as lymphokines) that are secreted by cells of the immune system

cytotoxic: toxic to cells

filamentous: adj. of filament, which is a single thread or thin flexible threadlike object, process, or appendage; esp. an elongated thin series of cells attached one to another (as of some bacteria)

genera: pl. of genus, which is a category of biological classification ranking between the family and the species, comprising structurally or phylogenetically related species or an isolated species exhibiting unusual differentiation, and being designated by a capitalized singular noun that is Latin or has a Latin form

granulomatous: of, relating to, or characterized by granuloma, which is a mass or nodule of chronically inflamed tissue with granulations (minute masses of tissue) that is usu. associated with an infective process

hematologic: of or relating to blood or to hematology, which is a medical science that deals with the blood and blood-forming organs [http://www.m-w.com]

hyphal fragment: part of a hypha, which is one of the threads that make up the mycelium of a fungus, increase by apical growth, and are coenocytic or transversely septate [http://www.m-w.com], plural – hyphae

macro: prefix – large

morphology: a branch of biology that deals with the form and structure of animals and plants esp. with respect to the forms, relations, metamorphoses and phylogenetic development of organs apart from their functions

mycelium: the mass of interwoven filamentous hyphae that forms esp. the vegetative portion of the thallus of a fungus and is often submerged in another body (as of soil or organic matter or the tissues of a host); also, a similar mass of filaments formed by some bacteria (as streptomyces) [http://www.m-w.com]

mycotoxicosis: poisoning caused by a mycotoxin

mycotoxin: a poisonous substance produced by a fungus and esp. by a mold

pathogenesis: the origination and development of a disease

pathology: the study of the essential nature of diseases and esp. of the structural and functional changes produced by them

septate: divided by or having a septum (a dividing wall or membrane)

somatosensory: of, relating to, or being sensory activity having its origin elsewhere than in the special sense organs (as eyes and ears) and conveying information about the state of the body proper and its immediate environment (~ pathways)

spore: a primitive usu. unicellular often environmentally resistant dormant or reproductive body produced by plants and some microorganisms and capable of development into a new individual either directly or after fusion with another spore

trichothecene: any of several mycotoxins that are produced by imperfect fungi (genera *Fusarium* and *Trichothecium*) and that include some contaminants of livestock feed and some held to be found in yellow rain [http://www.m-w.com]

Wall Cavity Samples
continued from page 61

Summary

Wall cavity sampling is often viewed as "looking for mold." But the real objective is to identify areas that either are, or were, affected by water intrusion. The mold may simply be the "marker" for the moisture damage. I consider wall cavity sampling to be an essential part of my consultant's "toolbox." Other than airborne samples, it is probably the most common type of sample that our staff collects. It can be used to identify areas that are currently wet, and those that were previously wet. It can be used to pre-select areas for destructive testing, prepare a scope of remediation, and assess the effectiveness of "restored" areas. These tasks can be accomplished at a reasonable cost and without disrupting the indoor environment.

Many water intrusions occur inside wall cavities, behind the toe kicks of base cabinets, and other inaccessible areas of a house. Wall cavity sampling is a cost-effective method for detecting such hidden fungal reservoirs. However, not every wall cavity or base cabinet should be sampled. Mold follows moisture, and sampling should also follow the moisture. Seven example locations were mentioned that are typically productive areas for sampling.

Collecting culturable fungi and fungal spores provides at least some information as to the relative age of the incident causing the mold. But, more important, collecting culturable fungi provides the opportunity to associate airborne exposures with hidden mold reservoirs.

Of course, the objective of any sampling method is to provide data that can be interpreted; the method has to have utility. The data obtained with wall cavity samples can be interpreted, by assessing the types of fungal spores that were detected, using numerical guidelines, and by calculating the ratio of culturable fungi to total spores.

Finally, what I consider to be the essential characteristics of a wall cavity sampling device have been presented.