

MICROSCOPIC EXAMINATION OF STICKY TAPE OR BULK SAMPLES FOR THE EVALUATION AND IDENTIFICATION OF FUNGI

By Chin S. Yang, Ph.D.

INTRODUCTION

During a building evaluation, signs of water damage or fungal growth often require laboratory confirmation and identification of causative fungi. A simple technique is to use a piece of clear sticky tape to pick up and remove suspect “fungal colony” for laboratory analysis. If no clear sticky tape is readily available, a piece of the material is removed for the purpose. A qualified, competent analyst trained in mycology and fungal identification can prepare and examine the sample for the presence of fungi, and identify the fungi. The results are qualitative and descriptive. However, the viability of identified fungi is unknown. Further tests are necessary to determine whether the identified fungi are viable or not.

This technical fact-sheet discusses various issues of using this technique during a building evaluation and investigation.

When and where to sample using the sticky tape method

The sticky tape method is useful and appropriate when you observe or suspect fungal growth on such surfaces as drywall, wallpaper, or ceiling tiles and would like to confirm the fungal growth, identify the types of fungi there, require a quick turnaround, and do not need to know quantities and viability of fungi.

How to take sticky tape samples

A detailed protocol is described in this fact-sheet (page 2). Please remember that this technique is for taking samples from smooth surfaces with signs of fungal growth. Do not sample from carpets or air duct liners. There are other sampling methods more appropriate for these materials. Do not sample from a desktop and expect the laboratory to give you spore counts.

What are the advantages of using the sticky tape method?

The sticky tape method is easy to use. It is relatively easy to analyze by experienced mycologists. The turnaround is relatively quick if not too many samples are taken and submitted. The major advantage is that fungi are observed and identified whether they are dead or alive. There have been cases where extensive fungal growth was observed but culturing failed to detect any fungi.

What the results mean

The following list is to assist you on result interpretation.

1. Bulk material and sticky tape samples taken for the direct evaluation of fungi and mold growth using optical microscopy provides qualitative assessment of fungal contamination and amplification. The most important assessment of this procedure is to determine whether fungi are colonizing, growing, and amplifying and to identify the fungi. Contamination is defined here as the types and/or numbers of fungal matter that are not normally there and should not be there. For example, *Stachybotrys*-like spores are normally not expected in a dry, clean indoor environment. Any detection of a *Stachybotrys*-like spore indicates contamination. On the other hand, *Cladosporium*-like spores are very common in any building; its mere detection does not suggest contamination. Amplification suggests fungal growth and reproductive increases in the fungal mass and number.
2. Results are qualitative and descriptive but do not indicate whether the observed fungal matter is viable, culturable or not.
3. The presence of a few loose fungal spores is considered as background, possibly spores in dust deposits.
4. The presence of spores and conidiophores suggests possible fungal contamination or growth, but spores and conidiophores can come from other sources or locations.
5. The presence of fungal hyphae, mycelia (aggregates of hyphae), and other fungal structures (such as rhizomorphs) suggests fungal colonization and growth (but not amplification because no spore is produced).

6. The presence of conidiophores (a spore-producing and -bearing structure), associated hyphae (vegetative fungal structures) and spores do suggest fungal growth and amplification.
7. The presence of spores does not necessarily indicate fungal amplification. The presence of an unusual number of spores of the same kind suggests fungal contamination from possible amplification sources nearby.
8. The degree of fungal growth and amplification is described as massive, numerous, many, a few and a trace. These descriptions are subjective and based on the analyst's experience and observation of the sample. Massive is used to describe a very heavy and dense concentration of fungal structures (whether spores, hyphae, or conidiophores, or any combination). Numerous describes heavy and dense concentrations of fungal matter, too numerous to count. Many is used to measure fungal matter that is heavy but countable. A few describe detectable and measurable fungal structures. A trace suggests that fungal matter and structures are barely detectable by an experience mycologist. It may be missed by less skilled analysts. No obvious fungal growth is clearly to describe no fungal growth observed, but loose background fungal spores and possibly hyphae may be observed. The following adjectives in decreasing sequence are used to describe various categories of growth: **Massive > Numerous > Many > A Few > A Trace > No Obvious Fungal Growth.**

Glossary:

1. Spores: a general term for a reproductive structure in fungi, bacteria, and cryptogamic plants. In fungi, spores may be sexual and asexual. Most indoor fungi are those producing asexual spores (or conidia), such as species of *Acremonium*, *Aspergillus*, *Alternaria*, *Penicillium*, *Stachybotrys*, *Ulocladium*, etc. Sexual spores are produced by Ascomycetes, Basidiomycetes, and Zygomycetes. Ascomycetes produce ascospores in asci. An ascus usually contains eight ascospores. Asci are often included in a fruiting body termed ascoma (an ascus-containing structure; pl. ascomata). Ascomycetes may be found growing indoors. Species of *Chaetomium*, *Eurotium*, and *Peziza* are frequently found on water-damaged paper or wood-products. Basidiomycetes produce basidiospores on a basidium. It usually has four basidiospores per basidium. A basidioma (pl. basidiomata) is a basidia-bearing fruiting structure. Several basidiomycetes may be identified indoors, particularly on wood structures indoors. Species of *Pleurotus*, *Sistotrema*, *Poria*, *Gloeophyllum*, *Serpula lacrymans*, *Coprinus*, etc. have been identified from badly water-damaged wood or paper-products in buildings. All these basidiomycetes are wood decay fungi. In most cases, basidiomycetes are identified from cultures or vegetative structures. Therefore, their true identities are often not known. Zygomycetes produce zygospores. Many zygomycetes are found indoors. They are: *Absidia*, *Choanephora*, *Cunninghamella*, *Mortierella*, *Mucor*, *Mycotypha*, *Rhizopus*, *Syncephalastrum*, etc. Zygomycetes often produce sporangia and sporangiospores. A few zygomycetes may produce conidia and conidiophores.
2. Because spores are propagules for dispersal, they are released individually or in clusters from a fungal colony. They may become airborne and then settle onto surfaces with dust. Therefore, any detection of loose fungal spores (unless some unusual spores, such as *Stachybotrys*-like, are detected) does not indicate fungal contamination. Only if fungal spores are attached to or associated with conidiophores and/or hyphae, then fungal contamination and growth are suggested.
3. Conidiophores: a modified hypha bearing or consisting of conidiogenous cells from which conidia are produced.
4. Hypha (pl. hyphae): a filamentous, vegetative structure of fungi. It is formed by a chain of fungal cells separated by septa. Some fungi produce modified hyphae that are characteristic to that fungal group. Clamped hyphae are characteristic of many basidiomycetes. Any observation of clamped hyphae suggests they are of a basidiomycete. Basidiomycetes may also produce modified hyphae, such as skeletal hyphae, binding hyphae, fiber hyphae, and skeletoid hyphae. The presence of these hyphae is indicative of basidiomycetes.
5. Mycelium (pl. mycelia): a mass of hyphae.
6. Rhizomorph: a root-like aggregation of hyphae. It provides functions similar to a root by absorbing and transporting water and nutrients. Basidiomycetes frequently produce rhizomorphs on and in substrates.
7. Pycnidium (pl. pycnidia): a more or less flask-shaped structure consisting of fungal tissues. Conidia and conidiophores are produced inside pycnidia. Species of *Phoma* produce their conidia inside pycnidia.

Protocol for Collecting Sticky Tape Samples for Fungal Assessment

Taking sticky tape samples for microscopic examination of fungal growth is a quick and easy technique if you have the tools listed below ready.

Obtain $\frac{3}{4}$ " or $\frac{1}{2}$ " wide clear sticky tape. The clear sticky tape may be found in a stationary store or in the stationary section of a large supermarket. If you have difficulty obtaining clear sticky tape, frosted tape, such as Scotch tape, is ok. **But never use clear packing tape or duct tape.** You also need labels or a marker pen to label your samples. Obtain some 1x3" microscope slides, aluminum foil or wax paper, and small slide boxes (clean plastic bags are acceptable).

Cut an approximately 3" long piece of tape and place sticky side of the tape onto the areas of suspected fungal growth. Gently press it to make good contact between the sticky surface and the "fungal growth." Remove the tape and observe to determine that the sticky surface of the tape has picked up some "fungal growth". Place it, sticky side down, on the glass slide or a piece of clean aluminum foil or waxed paper, folding the very end of the tape into a small tab or a handle. Mark your slide with a marker or a label. Put the slide in a slide box or a clean plastic bag. Repeat the process for additional samples. Make sure that you label and document each sample on your chain-of-custody sheet. Send a copy of the chain-of-custody with samples to the lab.

Sticky tape sampling is appropriate for smooth surfaces with visible signs of mold growth, such as drywall, wallpaper, ceiling tile, or wood. Do not use this sampling method on carpets or fibrous glass insulation. Bulk samples or vacuum dust samples can be collected from carpets or fibrous glass insulation for direct microscopic examination for fungi.

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