Microscopic Evaluation and Identification of Fungi From Tape-lift or Bulk Samples

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INTRODUCTION

Tape-lift or bulk sampling is an easy sample collection technique commonly used during a building assessment, when signs of water damage or fungal growth often require laboratory confirmation and identification of causative fungi. A piece of clear sticky tape is used to pick up and remove suspect "fungal colony" or "mold growth" for laboratory analysis. If clear sticky tape is not readily available, a piece of the "moldy" material (for example, dry wall) is removed for fungal evaluation. 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 to groups, genus or species. The results are qualitative and descriptive. However, this test does not determine the viability of identified fungi. Further tests are necessary to determine whether the identified fungi are viable or not.

This technical information document discusses various issues of using this technique during a building evaluation and investigation.

When and where to sample using the tape-lift method

The tape-lift 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 present, require a quick turnaround, and do not need to know quantities and viability of fungi.

How to take tape-lift samples

A detailed procedure is described below in this information sheet. Please remember that this technique is for taking samples from smooth surfaces with signs of fungal colony or mold growth. Do not sample from rough surfaces, such as carpets or air-duct liners by this method. There are other sampling methods more appropriate for these materials. Do not sample from a dusty desktop and expect the laboratory to give you spore counts.

What are the advantages of using the tape-lift method?

The tape-lift method is easy to use. It is relatively easy to analyze by experienced & competent mycologists. The turnaround time is relatively short 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 and confirmed by microscopic examination but culturing failed to recover any fungi.

What the results mean

The following steps are to assist you on result interpretation for microscopic evaluation.

1. Bulk material and tape-lift samples taken for the direct evaluation of fungi and mold growth using optical microscopy provides a 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 spores and matter that are not normally there and should not be there. For example, *Stachybotrys*-like or *Chaetomium* spores are normally not expected in a clean, dry indoor environment. Any detection of a *Stachybotrys*-like or *Chaetomium* 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 of spores.

2. The results are qualitative and descriptive but do not indicate whether the observed fungal matter is viable, culturable or not. However, a highly experienced analyst may be able to determine whether the observed fungal matter is viable or not by observations of fragmented hyphae and broken spores.

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 detection of mites, insects, small creatures, and their eggs and fecal matter is another indication of wet conditions. Furthermore, their presence is also an indicator of long-term growth of mold and fungi. Many mites and insects actually feed on fungal matter. This means that fungi have grown for a period of time before mites and insects are attracted to the food source.

9. Mites and insects forage on fungal growth and can contribute the fragmentation of fungal structures.

10. Nematodes, also known as roundworms**,** inhabit a very wide range of environments, including freshwater, marine, and terrestrial environments. They are very common in soils. Some nematodes are parasitic of plants and corps; and some are free-living in soils. We have found nematodes in samples from wet conditions.

11. Fungal growth stops when the water source is stopped and water activity is below the requirement for growth. A persistent desiccation will cause the fungi to die out over time. It may take a few months to a few years for them to die out, depending on the species and types of fungi. Dead fungal matter becomes brittle and breaks into pieces, when becoming dry. The observation and detection of fungal matter in pieces are excellent indications of old growth and dead fungal matter.

12. Fungal spread on the sample (whether bulk or tape-lift) is expressed by percentage of estimated growth coverage area. With the percentage and the size of the

sample, one can gauge the degree of mold growth on the sample. Fungal density is observed and estimated under the microscope. The scale 5 to 1 is used in decreasing order to indicate degree of fungal density, hence how robust the fungal growth was.

13. Because of taxonomical revision, we now group *Acremonium* and *Gliomastix* under the genus *Acremonium*. *Stachybotrys* and *Memnoniella* are united under *Stachybotrys*.

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 ascomytetes 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. 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 andconidiophores.

Because spores are propagules and designed 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 or *Chaetomium*, are detected) does not indicate fungal contamination. Only when fungal spores are attached to or associated with conidiophores and/or hyphae, are fungal contamination and growth suggested.

2. **Conidiophores**: a modified hypha bearing or consisting of conidiogenous cells from which conidia are produced.

3. **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 of 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.

4. **Mycelium** (pl. mycelia): a mass or aggregate of hyphae.

5. **Rhizomorph**: a root-like aggregation of hyphae. It looks like a root and functions like a root by absorbing and transporting water and nutrients. Basidiomycetes

frequently produce rhizomorphs on and in substrates. Many wood-decay basidiomycetes produce characteristic rhizomorphs, e.g. *Meruliporia incrassata.*

6. **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.

7. **Seta** (pl. setae): a stiff hair, usually thick-walled and dark in color. Some setae are very characteristic. For example, the basidiomycetes, *Asterostroma species*, produce star-shaped setae.

Procedure for Collecting Tape-lift Samples for Microscopic Fungal Evaluation

1. Taking tape-lift samples for microscopic evaluation of fungal growth is a quick and easy technique if you have the tools listed below ready. You may also obtain and use the pre-packaged tape-lift samplers available commercially. Follow the manufacturer's instructions if you decide to use the pre-packaged tape-lift samplers.

2. Obtain 3/4" or 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 1 x 3" microscope slides and small slide boxes (clean plastic bags are acceptable).

3. Cut an approximately 3" long piece of tape and place the 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 colony." Remove the tape and observe to determine that the sticky surface of the tape has picked up some "fungal colony". Place it, sticky side down, on the glass slide, 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.

4. 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.

5. Tape-lift sampling is only appropriate for smooth surfaces with visible signs of mold growth, such as drywall, wallpaper, or ceiling tile. Do not use this sampling method on carpets or dusty, heavily soiled fibrous glass insulation. Bulk samples or vacuum dust samples can be collected from carpets or fibrous glass insulation for direct microscopic examination for fungi.