

DIRECT MICROSCOPIC EXAMINATION

SAMPLE TYPES

- Tape lift (sometimes referred to as “sticky” tape or “transparent” tape)
- Bulk samples
- Swabs
- Wipes

PRIMARY PURPOSE

The primary purpose of a direct microscopic examination (wet mount) is to determine whether or not mold is growing on the surface sampled, and if so, what kinds of molds are present.

SECONDARY PURPOSE

Most surfaces collect a mix of spores which are normally present in the environment. At times it is possible to note a skewing of the normal distribution of spore types, and also to note “marker” genera which may indicate indoor mold growth.

- For example, when mold growth is present indoors, many more spores of a particular type will be found trapped on surfaces. In addition, these spores may be in forms which indicate recent spore release (close proximity), such as spores in chains or clumps.
- Marker genera are those spore types which are present normally in very small numbers, but which multiply indoors when conditions are favorable for growth. These would include cellulose digestors such as *Chaetomium*, *Stachybotrys*, and *Torula*. While a single *Stachybotrys* spore is occasionally seen as part of the normal outdoor flora, finding 5 or 6 of these spores on a single tape slide of a duct surface is an indicator that *Stachybotrys* may be growing indoors.

ADVANTAGES

A direct microscopic examination of a surface shows exactly what is there, without any skewing by laboratory procedures. Some spore types are able to thrive in a particular ecological niche but may not germinate at all on laboratory media. Fast growing spore types, or those with other competitive advantages may outgrow slow growing types or may prevent germination of others. Some spore reservoirs may be old and may have lost viability. These spores will not germinate and will be completely missed, even though dead spores are just as antigenic as live spores for allergic persons. There are times when cultures are helpful and will be necessary, for example, when speciation is desired for genera such as *Aspergillus* or *Penicillium*. But cultures of swabs or bulk samples should always be accompanied by a direct microscopic examination.

LIMITATIONS

Locating an area of mold growth indoors does not provide information regarding airborne spore levels. In order to cause health effects substantial numbers of spores must be inhaled by occupants, sometimes over a substantial period of time. Distribution mechanisms are an integral part of indoor mold problems along with sources of water and nutrients.

REPORTING:

- **AMORPHOUS DEBRIS:** Amorphous debris is an indication of the amounts of non biological particulate matter present. This background amorphous material is graded and described as scant, light, moderate, heavy, or very heavy. (Very heavy background debris may obscure visibility.)

- **MISCELLANEOUS MOLD SPORES AND/OR POLLEN:** Slides/specimens are examined for the presence of mold spores and pollen, noting the quantities and distribution of spore types found. A designation of “normal trapping” is made when a mix of spore types is present with the same general distribution as is usually found outdoors. In other words, the biological component of the sample surface is like that found everywhere. Types of spores present would include basidiospores (mushroom spores), myxomycetes (slime molds), plant pathogens such as ascospores, rusts and smuts, and a mix of saprophytic genera with no particular spore type predominating. (Many of these spore types would never be found growing indoors since many plant pathogens require living plants for growth, and mushrooms require compost, leaf duff of various types, or associations with roots of certain trees, etc. When a mix of spores seen include these types as well as pollen, the source is clearly the outside air, rather than indoor mold growth.) The numbers of miscellaneous spores seen are graded and described as none, very few, few, variety, and wide variety.

- **MOLDS PRESENT WITH UNDERLYING MYCELIAL FRAGMENTS AND SPORULATING STRUCTURES:** Slides/specimens are examined for the presence of mold growth, indicated by groups, clumps, and/or chains of single spore types, usually accompanied by intact mycelial and/or sporulating structures. These areas of growth are then identified to genus name, if at all possible. Quantities are estimated and are graded on a scale from 1+ to 4+, with 4+ denoting the highest amounts.

- **OTHER COMMENTS:** Additional relevant information is provided, such as the presence of marker genera or the abnormal distribution of spore types. Bacteria may be noted, as well as significant numbers of other biological particles such as algae, lichen, dust mites, etc. In addition, when deemed to be helpful, non biological particles are described.

- **GENERAL IMPRESSION:** A simple and concise impression is noted to assist the client with interpretation. These impressions are usually stated as “normal trapping” (the surface is not different from dust sampled anywhere), mold growth, possible mold growth in vicinity, no mold spores seen, etc.

REFERENCES

1. Beneke, E.S., & Rogers, A.L., *Medical Mycology Manual*, 3rd edition, Burgess Publishing Co., p.10, 1970.
2. Burge, Harriet A., *Bioaerosol investigations, in Bioaerosols*, Burge, H.A., Ed., Indoor Air Research Studies, Center for Indoor Air Research, Lewis Publishers, p. 17, 1995.
3. Macher, J.M., Streifel, A.J., and Vesley, D., Problem buildings, laboratories and hospitals, in *Bioaerosols Handbook*, Cox, C.S., & Wathes, C.M., Eds., Lewis Publishers, p. 513, 1995.

PROBLEMS WITH NUMBERS

Frequently, we are asked why we don't perform spore counts on tape slides, reporting results in spores/mm², or spores/in². Industrial hygienists and physicians often feel that numbers are easier to understand, more scientific, and more objective. In actual fact, we feel that with this type of testing numbers are less reliable and more subject to skewing than the visual observations of a skilled analyst. There are times when areas of mold growth are small and scattered on the tape prep. A simple count might very well miss the growth entirely, or provide an inflated impression of the amounts of mold

growth present. On any particular tape sample one can find a variety of places to count, providing a wide range of “counts,” which potentially could be very misleading. And, interpretation would still be needed as to whether the spores counted were all of one type or a mix of many. Finally, a direct microscopic examination (wet mount) can be performed quickly with a minimum of fuss and materials, making it possible to offer this test at a minimal cost. From a practical standpoint, more detailed and accurate information is available at less cost than time consuming counts.

PROBLEMS WITH CULTURES

Frequently, we are asked why we don't perform quantitative cultures on bulk samples, reporting results in cfu/gram of sample. Again, industrial hygienists and other safety personnel feel that numbers are easier to understand, more scientific, and more objective. And again, we feel that with bulk testing numbers are less reliable and can be very misleading. Protocols for quantitative cultures of bulk samples usually call for the grinding up of the sample (wallboard, wood, ceiling tile, etc.) in preparation for weighing. Serial dilutions are then made prior to plating. The question is, of what relevance is the weight of the sample material being cultured? Plaster, ceiling tile, wallpaper, wood, etc., will all provide very different weights. In most cases, the relevant information will be what is growing on the surface, since fungi are aerobic and will be sporulating on surfaces exposed to air. A careful examination under a stereoscopic microscope to locate areas of mold growth with subsequent direct microscopic examinations provides more accurate information at a reasonable cost. And, as previously mentioned, all fungi present will be seen and reported, including those which have lost viability, those unable to grow on laboratory media, and those unable to compete with faster growing, more dominant spore types.

Examples:

- Bulk sample #1: Carpet

Wet mount: 4+ *Torula herbarum*
3+ *Chaetomium* species
2+ *Ulocladium* species
1+ *Penicillium* species
1+ *Stachybotrys* species

Culture: 100,000 cfu/gm. *Penicillium* species
40,000 cfu/gm. *Cladosporium* species

- Bulk sample #2: Gypsum board

Wet mount: 4+ *Stachybotrys* species
3+ *Acremonium* species
1+ *Penicillium* species
1+ *Tritirachium* species

Culture: 120,000 cfu/gm. *Penicillium* species
10,000 cfu/gm. *Stachybotrys* species

Note: Samples shown were selected as examples of significant differences.

Exception: A case can perhaps be made for quantitative cultures of carpet dust, since by sieving (and other handling techniques) a gram of dust can be a comparable sample house to house, office to office. It is logical to assume that if spores from indoor mold reservoirs are finding distribution within an interior space, that the number and types of spores present per gram of dust would reflect this condition. Ginger Chew, a doctoral student at the Harvard School of Public Health, found a significant association between the presence of some culturable fungi in dust and in air.