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Sample and Analysis Types for Fungi and Mold
I. Culturable Samples

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The following is a short guide to the types of culturable samples analyzed by Fiberquant. It is not intended as an exhaustive sampling guide. Please refer to the following for sampling information: *Bioaerosols Assessment and Control.* American Conference of Government Industrial Hygienists. 1999. Sampling materials, such as culture plates, Ziploc bags, swabs, and tape may be obtained from Fiberquant.

Analysis of Culturable Fungi
Common to all analysis for culturable (sometimes called *viable*) fungi is that, at some point, an agar-containing petri dish is inoculated with possible spore or mycelium-containing material. That inoculation material may be part of a colony, an air stream, or a suspension in water. The resulting agar dish is incubated nominally at 25°C for 5-7 days, although incubation temperature and duration are dependent on the species of interest. Colonies are identified to genus or species, if possible, using a combination of colony macroscopic characteristics (*e.g.*, color(s), morphology, growth rate) and microscopic characteristics (*e.g.*, spore shape, size, color, hypha morphology). For quantitative methods, the colonies are enumerated as well as identified. Fungal growth will be reported as colony forming units (CFU). The term *units* is used since it is not known whether it was mycelia, one spore or many spores that grew into the colony.

Interpretation of cultured sample data is complicated by two factors: 1) certain species only grow well on certain agar media, and, therefore, may be present but not culturable because an inappropriate agar was used. Other species are not culturable at all. 2) Fungi grow at different rates, so slow-growing fungi may be over-run by fast-growing fungi, and, therefore, not be apparent in the culture. Therefore, to perform culturable sampling correctly, three or more types of agar may need to be used for each sample location.

Depending on client needs, the identification of colonies may be genus only, or speciated, if possible. Generally, speciation is attempted on cultures having only one colony that can be allowed to grow (bulk), while speciation will not be attempted on sample expected to have numerous types of fungi that must be identified while their colonies are still small (Andersen, Swab, Dust).

1. Bulk Sample
A bulk sample is a piece of a fungal colony. It may include the matrix (*e.g.*, a piece of wallboard), or not (*e.g.*, a tape lift). The purpose of this type of sample is to identify the fungus. To sample, collect what appears to be one kind of fungus. Many infestations consist of multiple colonies – these must be sampled separately, for the reasons stated above. For a matrix sample, merely collect part of the colony and place it in a Ziploc bag. For a tape lift sample, touch the middle of a 3" x0.75" piece of tape (such as 3M Crystal Clear) to the colony. The deposit should be heavy enough to be easily seen on the tape. Adhere the tape to a clean glass slide. The middle may not adhere due to the deposit, but the ends will stick. In the lab, some of the bulk material will be used to inoculate an agar petri dish, and the resulting colony identified, as above. The results will be qualitative: the identification of the type(s) of fungi observed.

2. Direct Deposition (Andersen) Sample
The Andersen or equivalent sampler directs an air sample through 400 drilled holes. The spores or other fungal material impinge directly onto an agar petri plate. See the above reference or pump literature for specific sampling instructions. The resulting agar dish is submitted to the lab already inoculated by the pump. After incubation, the colonies are counted and identified to genus level, if possible. In addition to the general caveats of culturing given above, the Andersen-type sample has two others. A maximum of 400 colonies per sample can be counted, but a colony could have been formed from one spore or a group of a hundred, so Andersen-type samples may not be indicative of numbers of spores (or concentrations of mycotoxins, which would be dependent on numbers of spores). Also, if two different spores go through the same hole, only the fastest growing colony will be counted, so volumes on Andersen samples must be kept low to minimize such occurrences.

3. Swab Samples
Wetted swabs may be wiped across a known area to pick up fungal contamination. Swabs are not a good way to collect a bulk sample (rather use one of the techniques mentioned in 1. above). In the laboratory, the swab will be ultrasonicated to suspend any sample particles in water, and then a portion of the suspension is cultured. A series of cultures are made, each with $1/10^{th}$ the amount of the original as the previous culture, so that one culture plate is obtained that has a suitable number of colonies to be counted and identified. The results will be given in CFUs/cm2. The efficiency of the swab sampling process is unknown, as is the efficiency of spore suspension, so swab samples are best performed as a comparison between areas of possible contamination and areas of known non-contamination.

4. Dust Samples
A dust sample is a macroscopic (weighable) amount of dust. It may be collected directly, or collected with a vacuum or pump system. In the laboratory, a sub-sample is weighed to 0.00001 gm, suspended in water, and then a portion of the suspension is cultured. Like the swab sample above, a series of cultures are made from dilutions of the original suspension, so as to obtain a culture plate suitable for counting and identification. The results will be given in CFUs/gm.