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Sample and Analysis Types for Fungi and Mold
II. Non-Cultured Samples

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The following is a short guide to the types of non-cultured 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 filter cassettes, microscope slides, Ziploc bags, and tape may be obtained from Fiberquant.

Analysis of Non-Cultured Fungi Samples
As opposed to cultured-type (sometimes called *viable*) analysis, in which an agar-containing pitri dish is used to grow colonies, non-cultured-type (sometimes called non-viable) analysis involves direct analysis of the fungal material present in the sample. The material may be growing on a substrate, captured on tape, or captured by an air sampling device. In order to analyze such samples, the sample or sub-samples are mounted on a microscope slide(s) to be examined at 200x-1000x (magnification depends on the type of sample). Spores and other fungal structures are identified to the genus level, if possible, by optical characteristics (*e.g.*, spore shape, size, color, hypha morphology). The spores identified or counted may or may not be able to germinate (thus the name *non-viable*)

Interpretation of non-cultured sample data is complicated by two factors: 1) Usually, identification must be made from a spore or spores alone. In some cases, this is enough information to identify the genus (*e.g.*, *stachybotrys*). In other cases, spores from different genera look so similar that they cannot be distinguished (e.g., *penicillium* and *aspergillus*); therefore, these must be reported in a combined category (*e.g.*, *penicillium/aspergillus*). 2) Spores are not the only fungal material observed in samples. Mycelial fragments occur unassociated with spores, and cannot be further identified except as mycelial fragments. In spore trap samples, the mycelial fragment count does not contribute to the total spore count. In bulk samples, mycelia that cannot be associated with some spore type are also reported separately, but, in this case, contribute to the total % fungus.

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 should be sampled separately, since the presence of one may hinder the visibility and ability to identify another. 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 mounted on a microscope slide, if not already, and the resulting spore types identified, as above. The results will be semi-quantitative: the identification of the type(s) of fungi observed and the estimated percentages of each. Swabs generally should not be used for bulk sampling, since most of the material becomes caught in the cotton fibers.

2. Spore Trap (Zefon) Sample
Even though there are other types of spore traps, the Zefon is the most common, and the one for which our reporting system is optimized. Spores (and any other debris) are trapped as an air sample impinges a sticky surface. Typical volumes are 75-150 L, corresponding to 5-10 minutes sampling. Sample volumes from areas expected to be spore-laden (e.g., infestation rooms, moist climates) should be limited to 75 L. Samples of typical outdoor Arizona air should be 150 L. Samples from behind walls should be 7.5-15 L (or 1 to 2 minutes sampling time). In the lab, the cassette will be disassembled, and the inner cover-slip-like piece that holds the sticky surface is removed and mounted on a glass slide. A drop light stain is added to emphasize colorless spores and to exclude air from the mount. Spores are counted as the thin strip of debris is scanned back and forth. Nominally, fifteen such passes across the strip are made. Results will be (for each category of spore and total) raw counts, % of each category of spore, and counts/m3 of air.

3. Tape Lift (Analyzed per Area)
This type of sample might be used to check for contamination. In this case, the tape is touched once only to the surface (preferably a smooth surface, although fabrics have been tested like this also). The tape lift must be put on a clean slide in the field, since further manipulation can only invalidate the sample. In the lab, a certain area of the tape will be analyzed, nominally 50 fields of view. The results will be in spores/cm2 of tape.