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Spore Trap Counting Methods

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As of the current date, there is no published or standard method for counting spore trap samples. Therefore, each laboratory has developed its own method for counting, and great variation may be expected between labs as they each count different percentages of the deposit, and even use different instrumentation and magnification. This circular discusses Fiberquant's adopted methods. (The examples below are specific to Zefon spore traps; the details would differ slightly for other types of samples.)

The Spore Trap Problem
Spores may be as small as 2um diameter or as long as 30 um. To see detail on a 2 um particle (e.g., smooth or rough, point of attachment, *etc.*) that may be required to distinguish spore types, the highest resolution available on an optical microscope is required, normally 1000x magnification using a 100x oil immersion objective. Ideally, the entire deposit would be examined at this magnification. However, the cost for such an analysis would be overwhelming. One compromise which can be made is to count a portion (20-25%) of the deposit. This has the advantage that any spores observed may be identified at the best resolution, but the disadvantage that clumps or strings of spores inhomogeneously distributed over the deposit will cause this type of analysis to be less reproducible than an analysis which counts the entire deposit. A different compromise which can be made is to count the entire deposit, but use a magnification (400-600x) which allows the analysis to be completed in an economically feasible amount of time. The advantage to this compromise is that its reproducibility should be high (depending on the skill of the analyst). Its disadvantage is that certain spore types which may be distinguished at 1000x may not be distinguishable at 400-600x. Another complicating factor is that 40-60x objective lenses (normally yielding 400-600x) are usually dry or air immersion in use, whereas the resolution desired at 1000x can only be achieved using a 100x oil immersion lens (100x dry lenses exist, but do not produce any better resolution than a dry 60x lens). Therefore, if one is scanning using a dry 60x lens, one cannot very well change to 100x oil, then go back to dry conditions. Once oil is on the slide it is difficult to clean off.

Fiberquant Analytical Services Approach
Recognizing that oil immersion is a requirement to the best resolution and best spore differentiation, Fiberquant uses oil immersion objectives for spore trap analysis. Also recognizing that medium magnification may have advantages in spore trap analysis, we have purchased for each microscope a 50-60x oil immersion objective as well as the usual 100x objective, allowing the analyst to readily change back and forth from 500-600x to 1000x, all in oil immersion mode, as required by the demands of the analysis. Since there currently is no published procedure, Fiberquant offers three types of spore trap counts, ranging from a scan of the entire deposit at 500-600x, a scan of a portion of the deposit at 1000x, and a combination of the two, which somewhat captures the best of both magnifications. These are described below.

In all spore counts, the interpretation of spore trap 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-like*). 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 and are reported separately

SPCT
This is a count of 15 passes across the deposit (approximately 20%) at 1000x only, as our analysts were taught at McCrone Research Institute. In the absence of a published method, since a respected teaching center recommends this procedure and magnification, this is a defacto standard method. (Since SPCT2 also incorporates 15 passes at 1000x, it can be considered an enhancement of this method.) Every fungal particle observed during the passes is counted and categorized. Both the spores/sample and the spores/m^3 are calculated numbers, rounded to two significant digits. The analytical sensitivity (hypothetical observation of one spore per analysis) is 65-71 spores/m^3 for a 75L sample, depending on the microscope used.

SPCT1
This is a scan of the entire deposit at one magnification only (500-600x oil immersion). Every fungal particle observed is counted and categorized, although in heavily loaded samples, some smaller spores (*e.g.*, *Pen/Asp-like*, basidiospores) may not be observable at this magnification. Unless the deposit is highly loaded, each spores/sample is an exact count, reported to 1 spore; each spores/m^3 is a calculated number, rounded to two significant digits. The analytical sensitivity is 13 spores/m^3 for a 75L sample.

SPCT2
This method uses both medium and high magnifications. A scan of the entire deposit is performed at 500-600x oil immersion, during which large spores, pollen, fragments, and clumps or clusters of spores are counted. Then, 15 passes across the deposit are performed, during which the small spores not in clumps or clusters are counted. The results from the two counts are mathematically combined into one result for each spore type. For a spore type expected to be reliably counted during the full scan, its spores/sample result is a count, reported to 1 spore, and its analytical sensitivity is 13 spores/m^3 for a 75L sample. For a spore type expected to have counts at 1000x, its spores/sample result is a calculated value, reported to two significant figures, and its analytical sensitivity is 65-71 spores/m^3 for a 75L sample.