Labs and Testing: Inter-laboratory Variability in Spore Trap Analysis

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The two main methods used for microbial air sampling today are culturable and total spore air samples. The culturable method, which collects spores on an agar plate, has been used for over 150 years. Similarly, spore impingement on a glass slide dates back to the 1870s. For each of these sampling methods, the samples are analyzed by a laboratory and a trained microscopist.

Most of the time, we assume that the data from the lab is an exact and reliable number. However, we need to remember that any sample is a statistical approximation, fraught with both quantifiable and unquantifiable errors. First, the equipment introduces errors when a sample is taken. This is sampling error, or SE. Second, there are analytical errors introduced when the technician analyzes the slide. This is analytical error, or AE. Both types of errors can be summed together into the SAE, or sampling and analytical error of a sample. This SAE is rarely quantified or even considered in reporting microbial sampling results to clients.

First, let’s tackle sampling error. When taking air samples, it is important to realize that flow rates are never held perfectly constant. Measurements of the sample volume are dependent upon the accuracy of the flow calibration device and the timer. These errors in the sample volume measurement contribute to the SE, and have a variance of less than 10 percent with the use of a primary standard calibration device.

A second component of the sampling error is one of collection efficiency. The collection efficiency of mold spore sampling devices varies considerably and is highly dependent upon the size of the mold spores being sampled. The collection efficiency of the various spore trap samplers is still something of considerable debate.

Once a spore trap sample is collected and sent off to the lab with a reported flow rate and understood collection efficiency, the laboratory has to analyze the sample under the microscope. This process introduces a certain amount of analytical error.

Published research has not compared inter-laboratory variation on the identification and interpretation of total spore sampling results, nor has research calculated the SAE. This is not surprising, since each laboratory has its own identification and counting methods for spore traps.

Some indoor environmental consultants justify ignoring the issue of the analytical error in spore trap analysis, claiming that the "normal" variation in the concentration of total mold spores in the air far exceeds the variation introduced at the laboratory. This assumption is not based on the scientific research. It is also inconsistent with published, long-term microbial studies of controlled indoor environments. Granted, there can be a large variation in indoor mold spore concentration when mold spores are disturbed, but most monitoring is done prior to or after mold spores have been disturbed.

Spore trap analysis is highly dependent upon the visual skills of the microscopist in both identifying and quantifying the mold spores and other materials. In fact, this source of error is probably most significant. If the analyst misidentifies or undercounts the mold spores, misinterpretation and erroneous conclusions could be drawn from their analysis.

How significant is this spore trap analytical error issue? Testing this question is difficult. It is highly dependent upon the level and types of mold spores in the air. If one has low levels of big spore genera, it is easy to count the slides. Conversely, if one has high levels of small spore genera, it can be very difficult and time-consuming to adequately count the slides.

An interesting aspect of this counting question is that many commercial labs typically allow the microscopist six to eight minutes to count a spore trap slide. Ironically, for counting fibers for asbestos monitoring, which is much simpler, NIOSH specifies at least 10 minutes for counting each slide.

This research study has concluded that a majority of the SAE is a result of analytical error at the laboratory, not sampling error on the jobsite.

Spore Trap Counting Variability Research

Project

During the summer of 2006, my company encountered an environment that was contaminated with mostly Aspergillus niger. These spores are relatively small, which makes them an ideal test for lab counting variability. The presence of Aspergillus niger was identified in the first round of sampling that included four culturable air samples and four total mold spore samples. The building owner allowed us to come back and do a second round to evaluate the question of inter-laboratory variability in spore trap counting methods. He was not occupying the space and had an interest in research. At the same time, a number of laboratories agreed to volunteer their analysis services. Without the help of all these companies and individuals, this research would not have been possible.

In this research project, four spore trap sample slides were prepared and read by the first laboratory. These same four slides were then sent to six other laboratories starting in August 2006.

This research evolved into two projects. This first project focuses on analysis error, and identifies the laboratory variation in the analysis results. A second research project will focus on sampling error and collection efficiency of four different spore trap devices including the Air-O-Cell, Micro 5, Allergenco-D and Cycle D spore traps. Analysis of 28 samples for the second project is still in progress.

After the initial mounting and analysis of the four slides in this first project, each laboratory knew it was doing a recount as part of a research project. It is therefore likely that they used a more experienced microscopist to count these slides. In fact, if I ever send a set of slides for a second opinion, I would expect the second laboratory to use a more experienced microscopist.

Each volunteering laboratory had a different report format. Some included additional analysis for algae, hyphae, insect fragments, etc. The comparable analysis data for particular mold genera were extracted from the various lab reports and are shown in Tables 1 through 4.

As one can see in the tables, there was considerable variation in each analysis of the spore traps. Not only was there significant variance in the counts, but there was differing interpretation as to the identification of the genera.

For example, some labs identified some of the less frequently

found spores while others did not. The only mold genera for which there is fairly consistent data from all six labs are *Cladosporium* and *Penicillium/Aspergillus*-type spores.

Obviously, the high variability of the data indicates that many complex factors are involved in spore trap counting. For example, where the *microscopist* views the trace appears to make a difference. In Table 2, two labs found some *Trichoderma*. It appears this genus was only in one spot on the trace, and these two labs happened to view that portion of the trace. A similar condition appears to have been present for *Nigrospora* in Table 3.

Realistically, there is so much variance in this data that little statistically useful information can be gained by a detailed analysis. However, some limited information can be gained by simplifying the information in the tables.

The first simplification that we can make in analyzing these results is just to look at the number of spores counted, rather than the concentration of spores in the air. We can do this because the sample volumes were all equal. The volume of air sampled is just a mathematical adjustment to the actual spore counts.

The second simplification would be to initially analyze the variability in counting the most common or frequently encountered mold spores, such as *Cladosporium* and *Penicillium*/*Aspergillus*-type spores. The assumption here is that since these are the most common genera encountered by the laboratories, the variability in identification and counting should be less than for other genera.

These two simplifications were used to develop Tables 5 and 6. Table 5 shows the *Cladosporium* spore counts for all four slides, while Table 6 shows the *Penicillium*/Aspergillus-type spore count data for all four slides. Each table also shows the statistical *parameters* of the average spore count and the standard deviation. The standard deviation is a statistical measure of the variance in the data.

Lastly, the standard deviation is shown as a percent of the average. This is known as the relative standard deviation. For a normal distribution, the relative standard deviation is 33 percent of the average. Here, the relative standard deviation is at least 50 percent of the average. Therefore, the data are not normally distributed.

For a normally distributed data set, adding or subtracting one standard deviation from the average of a data set will give you the numerical range for 66 percent of the data. Adding or subtracting two standard deviations from the average will give you the numerical range for 95 percent of the data.

For example, for sample 1 in Table 5, the average *Cladosporium* spore count is three spores. Adding one standard deviation to the average gives a maximum spore count of 4.5, while subtracting one standard deviation from the average gives a lower spore count of 1.5. This means that 66 percent of the lab counts were between 1.5 and 4.5. Adding two standard deviations to the average gives a maximum spore count of 6.0, while subtracting two standard deviations from the average gives a lower spore count of 0. This means that 95 percent of the lab counts were between 0 and 6.0.

What this research is trying to quantify is that given a specific laboratory result, how much variation in that number can be expected? For example, based on Table 5, should the laboratory then report *Cladosporium* results as plus or minus 50 percent? Further, based on Table 6, should one report *Penicillium*/*Aspergillus* results as plus or minus 72 percent?

So, which lab result is actually correct? Is a higher spore count the most accurate, or is it possibly misidentified and over counted? For example, the uniqueness of *Cladosporium* spores makes misidentification unlikely. A number of other factors can lead to higher spore counts, probably the most significant of which is whether the slide was examined at 400× or 600×. There is good scientific evidence showing that increasing the level of magnification during examination of a spore trap slide will yield higher spore counts. A second factor is the wideness of the field of the objective lens of the microscope. With a wider field, the *microscopist* can see a larger area and, hence, more spores.

A third factor is that most labs read about 25 percent of the trace and then multiply that count by four to estimate the total count on the trace. Many also read up to 100 percent of the trace looking for genera with a single-digit spore count. Reading more of the trace can result in more accurate, higher counts. A fourth factor is the visual acuity of the *microscopist*. Some people are able to see finer detail because they have more cells in their retinas. Fifth, experienced *microscopists* are more likely to properly identify and count spores than novice readers.

Could one have sent these slides to a laboratory and had the whole slide counted to get the "correct" count? At what magnification, etc.? The problem with having this done is that it is not a normal analysis method. This project was to see how much variability exists in the methods currently used by laboratories.

Based on this data, one could draw the conclusion that, most of the time, spore counts are undercounted. This would mean that one should typically report *Cladosporium* results as possibly being up to 50 percent higher and *Penicillium*/*Aspergillus* results as being up to 76 percent higher. In our data, this assumption was accurate half the time.

Clearly, this research raises serious and significant questions about using only spore traps to evaluate and classify the mold spore character of an environment. It also raises serious questions as to the scientific accuracy of using only spore trap data in legal cases.

Recommendations for Indoor Environmental Consultants and Analysis Laboratories

Based on this research, a few recommendations for consultants and analysis laboratories are provided below:

Laboratories should offer a "replicate" spore trap analysis service for legal cases. This service would use two microscopists to evaluate each spore trap slide. The results of the two microscopists would then be reported as a range. This would better quantify the variance associated with visual identification and give the IEP higher confidence in the data.

Laboratories should consider establishing a special "recounting" or "expanded" counting service. This service would involve counting a much larger portion of the slide. For example, one technician would read one area, and the other technician would read a different area. A much greater portion of the trace would be read in this manner. The special analysis results would be reported with a variance (e.g., plus or minus 25 percent) for the counts for each genus.

Laboratories should notify clients whenever clusters of spores

were present. In this study, one lab reported clusters as potentially being the reason for such high variability amongst the labs. Clusters provide one of those time consuming counting challenges. Also, clusters can be broken up in Andersen samplers and result in higher reported culturable levels.

Lastly, we have undertaken a much broader study in the variability of spore trap counting along with various types of spore trap samplers. This study involves 28 slides taken at three different spore concentrations with four different types of spore trap media. The results of this research will be published later this year. If you are with a laboratory that wants to participate in this study, please contact me.