A LARGE SCALE STUDY OF REAL WORLD COMPARISSONS

(*Originally Published in the November 2007 issue of Indoor Environment Connections*)

In theApril, 2007 issue of IE Connections, we presented the results of a research study on the Inter-laboratory Variability in Spore Trap Analysis. That research study involved seven different AIHA EMPAT- "proficient laboratories reviewing the same four spore trap slides collected in a mold-contaminated condo. A comparison of the laboratory analysis showed significant variability of at least 50-75 percent in the reported spore counts.

This significant analysis variability occurred even for common spores types, such as cladosporium and penicillium/aspergillus. This raised significant questions about the interpretation of spore trap results.

Given the critical importance of spore traps to the indoor air quality industry, we felt the question of inter-laboratory analysis variability needed to be further researched Of particular interest was whether different spore trap sampling media had any effect on this amount of variability. For example, were some spore trap slides or spore deposition pattens easier to read than others?

Consequently, a second large-scale research project was designed to measure what effect the use of different spore trap samplers would have on the variability of reported sampling results.

This study evaluated four types of spore trap samplers at three different spore and debris concentrations. There were two sets of each indoor sample and one set of outdoor air samples. This generated a total of 28 spore trap slides.

Seven laboratories agreed to participate in both studies, with the results being presented anonymously. The labs included EMLab P&K at the time, they were two labs - Aerotech and EMLab, QLab, QuanTEM, Northeast, Aerobilogy and EMSL.

Without the contributions of These laboratories, this research would not have been possible.

Environmental Monitoring Systems donated hree types of spore traps, including Micro 5, Cyclex D and Allergenco D, for this research subject, OEHCS provided the Air-O-Cells, sampling equipment and research analysis. We wish to thank the condo owner for allowing us to conduct this research prior to remediation of the interior space.

The sampling was done in August, 2006. Logistically, it took almost one year for the laboratories to review all 28 slides. The sampling properties of the four samplers are shown in Table l. The cut-off diameter is the aerodynamic diameter of the airborne panicle at which the collection efficiency drops to less than 50 percent.

The aerodynamic diameter is a function of the physical size, shape and density of the particle. This means the aerodynamic diameter of the particles (in this case, mold spores) of less than the cut-off diameter are collected less than 50 percent of the time. This collection efficiency decreases further with smaller diameter particles.

Conversely, the collection efficiency increases with large-size particles.

Most mold spores range in diameter from 2 to 20 microns. Some are smallen Some are even larger, especially when clumps of spores are present Also, mold hyphae and other biogenic particles can also be smaller than two microns.

Hence, the more recently developed spore trap samplers have been designed with a lower cut off diameter in an attempt to capture smaller mold related biogenic materials. The use of different spore trap samplers with varying cut off diameters would test weather this makes a difference in real world sampling conditions.

Sampling Apparatus

The four different spore trap samplers were mounted next to each other in a 2.5x2.5 inches square sampling area. (four circular media mounted next to each other) All the air inlet holes were within 2.5 inches of each other. This meant that they were drawing air from essentially the same air space. The various samplers were connected to different sampling pumps and calibrated to the manufacturers recommended flow rate. All samples were colected for 5 minutes

Sampling Conditions

The indoor sampling conditions were varied by changing the amount of airflow in the condominium The three airflow conditions were:
1. Low Concentration - quiescent air How;
2. Medium Concentration - window fans operating at door level, and;
3. High Concentration - window fans operating at floor level with a leaf blower directed at the water-damaged door and wood ceiling.

The indoor temperature was 72 F with a relative humidity of 38 percent at the time of testing.

Outside conditions were 70 F with wind speed less than 5 miles per hour.

Sampling Locations
Three sampling locations were chosen. The two indoor sampling locations were the living room (Area 1) and the open loft bedroom above the living room (Area 2). 'I`he outside samples were taken on the outdoor balcony.

Sampler Validation

In some respects, this research project also became a real-world validation study of four types of spore trap samplers. As a perspective, most validation studies of sampling devices are done under controlled laboratory conditions. Few are ever done using real-world sampling conditions. Furthermore, most validation studies only use one analysis lab and base comparisons solely on these labs' results. This validation study was based on the comparison of the analysis of the spore traps from seven different laboratories, making this study's results far more significant.

Sampling Results

Each lab reported over 30 different genera of mold spores in addition to other biogenic categories such

as algae, hyphae, insect fragments, mildew, etc. All this data was entered into a spread sheet for analysis. The results showed significant variance both in spore counts and spore identification.

The only mold genus for which there was fairly complete data from all seven labs was cladosporium. lt was reported 98 percent of the time on the same slides.

Penicilliumlaspergillus type spores and basidospores were reported 80 percent of the time on the same slides. The reporting consistency percentage further decreased for smutslmyxomycetes/periconia (77 percent), ascospores (67 percent), alternaria (63 percent) and pithomyces (51 percent).

All other spore genera had less than 50 percent reporting consistency. It was initially envisioned that all analysis results of the 28 spore trap slides from the seven laboratories would be printed as part of this article. However the tables would have taken up many pages of print space. The complete spreadsheet containing all data can be obtained from the author via email. Given this significant lack of consistency in spore identification, it was decided to focus on the most consistent data. This was Total Spore cladosporium and pen/asp spore counts. These data were extracted from the main spreadsheet and are shown in tables 2-5. The tables are grouped as Low, Medium, High and Outdoor concentration, based on the sampling conditions and locations discussed above.

Analysis of the Sampling Results

Tables 2-5 show considerable variation between each laboratory's analyses of the spore traps. Further the amount of variability was the same or greater than that shown in the previous research study. The first look at the laboratory analysis results showed the low and medium samples were of the same magnitude, with only the high and outside samples being significantly higher. Consequently the low and medium sampling results were combined for analysis purposes. Evidently it took the high velocity air from the leaf blower to significantly aerosolize settled mold spores and generate elevated levels. This finding has potential implications regarding the need for aggressive air washing as part of mold remediation.

Low Medium Total Spore Concentration Samples

Tables 6-7 show the low/medium total spore analysis results for the various samplers. The reported results were highly variable with the relative standard deviation ranging from 15 to 123 percent. In this case the Cyclex D gave the highest counts in three out of four samples with the Air-O-Cell coming in second highest. This difference was not statistically significant (see below).

However, total spore count results are very limited in their usefulness in interpreting mold spore levels in indoor environments. More use ful to IEPS are the levels of the individual mold genera or groups, which we next consider next.

Tables 8 and 9 show the cladosporium spore concentrations by sampler types. Cladosporium is the most common mold spore genus in the world. It is also the genus one would expect all EMLAP laboratories should consistently and accurately identify. As shown in the tables, the Cyclex D again gave the highest average results in two of four samples. On the other hand, the Allergenco D gave the lowest results in three of four samples.

This difference was not statisti- cally significant (see below). The results were highly variable with the relative standard deviation ranging 67-110 percent.
We also determined whether similar results were reported for pen/asp spores. Tables 10 and 11 show the pen/asp spore concentrations by sampler type. Pen/asp spores are indicator mold generas, meaning that elevated levels of these types of spores are associated with water or moisture intrusions.
For pen/asp spores, the Air-O-Cell gave the highest results in three of four samples, with the Allergenco D again giving the lowest results in three of four samples. This difference was not statistically significant (see below).

The results were also extremely variable with the relative standard deviation ranging 70-194 percent. These results are different from the total spore and cladosporium results because of higher variability.

High Concentration Sample Results

The high concentration sample results are shown in Tables 12-14. They show Total Spore, Pen/Asp Spore type spore and Cladosporium Spore concentration. The high-concentration samples were high in both spore counts and debris rating (4+). Because of the high debris levels only about half the labs actually analyzed the high level spore trap slides. Normally, labs state in their reports that with high debris rating (4+), the actual spore counts are higher than those reported. Consequently, the levels in these tables should be viewed in this perspective, with prob able concentrations being actually higher

Outdoor Sample Results

Tables 15-17 show the outdoor air sample spore analysis results. The outside samples had relatively high spore concentrations with low debris levels. These slides were therefore much easier to read, but required more counting by the microscopist. The outdoor results were not quite as variable as the indoor results, with the relative standard deviation ranging from 15 to 95 percent. As opposed to the indoor air samples, in this case the Allergenco D had the highest results in two of three samples with the Cyclex D results showing the second highest.

Statistical Analysis

All the above laboratory results show high variability in spore quantification. Given this high variability, is it possible to differentiate whether more of this variance is due to the sampling media or the lab analysis? Given the large amount of data and the variation in the numbers, the only way to determine scientifically is to mathematically analyze this data statistically. There are many types of statistical analysis methods. All are based on assumptions on properties of the data set, whether they are a probability disuibution, logarithmic, linear, etc. We chose linear regression and Student's T test methods.

Counting Variability Differed by Spore Type

One very interesting statistical trend, that the relative standard deviation was significantly higher for pen/asp spores than for cladosporium spores, was present in all the sample sets. This same trend was also shown initially in Research Project #1. The fact that this trend also appeared in this much larger study says something about pen/asp spore counting versus cladosporium spore counting. Table 18 shows the average Relative Stan- dard Deviation for Total Spore, cladosporium spores and pen/asp spores for the various concentration ranges. This table shows that pen/ asp spore counting is 25-40 percent more variable than cladosporium spore counting. This is

true even though the average concentration for each spore type was approximately the same, meaning the microscopist was counting the same number of spores for each genus. These differences in variability are statically significant at the P <0.05 level.

Does this indicate that lab technicians have more difficulty identifying and counting pen/asp spores than cladosporium spores? Since correct quantification of indicator species such as pen/asp is so important, the reasons for this increased level of variance should be further researched

Variability Decreased With Increasing Spore Concentration

It should also be noted that, in Table 18, as the spore counts increased, the variability in spore counts decreased. This decreasing variability normally occurs with increasing sample size. Therefore this decreasing variability is normal.

Minimal Effect From Different Cut-Off Diameters or Different Samplers

As discussed above, each of the samplers had a different cut-off diameter. Since different cut-off diameters should result in different collection efficiencies, one would have expected to see some effect in the reported lab results due to this variable.

For example, it would seem logical to assume that the sampler with the lowest cut-off diameter (Micro 5) should have produced, at least, slightly higher sampling results. However, the data did not show this, possibly because small spores were not present in this condo.

Also noted above, the Cycle D had the higher numerical results for many of the low/medium indoor samples. On the other hand, for the outdoor samples, the Allergenco D had the higher numerical results for two of three samples with the Cyclex D as the second highest.

A linear regression analysis was performed to compare sampler type to total spores, cladosporium spores and pen/asp spores concentrations. The analysis showed that because of the high variability in the lab results, no statistically significant difference was found between the four types of samples for Total Spore and cladosporium concentrations.
On the other hand, two studies in the published literature show a difference in collection efficiency based on laboratory testing.

These are “Collection of airbome spores by circular single-stage impactors with small jet-to-plate distance” (S.A. Grinshpun, G. Mainelis, M. Trunov, R.L. Gémy, S.K. Sivasubramani, A. Adhikari, T. Reponen, Journal of Aerosol Science, 2004)

and

“A small change in the design of a slit bioaerosol impactor significantly improves its collection characteristics” (Sergey A. Grinshpun, Atin Adhikari, Seung-Hyun Cho, Ki-Youn Kim, Taekhee Lee and Tina Reponen, Journal of Environmental Monitoring, 2007).

At a future date, we will conduct additional statistical analysis of these data by individual laboratory. It may be that some laboratories were able to tabulate a difference in the individual samplers and their collection efficiency. We will also rank the laboratories by their individual variability to see if there was any difference.

Lastly, further statistical analysis of these data will also look at the variability in the analysis results by individual sampler.

Conclusions

This research project confirms that the laboratory analysis results, even among EMPAI1 proficient laboratories, are highly variable. This variability increases from common to uncommon spore types. For example, pen/asp spore counting was 25-40 percent more variable than cladosporiurn spore counnng with this difference being statically significant at the P <0.05 level.

Further, because of this higher variability, the average laboratory results of this study were not sensitive enough to show any statistical difference in the results from the four types of samplers tested.

Clearly, spore trap analysis is highly dependent upon the visual skills of the microscopist in both identifying and quantifying the mold spores and other materials. If the analyst misidentifies or undercounts the mold spores, misinterpretations and erroneous conclusions could be drawn from the analysis.

This human error factor is critical in spore trap analysis. This raises 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 spore trap data in legal cases.

Recommendations

This research clearly identified the need to determine why so much variation exists in the quantification and identification of mold spores on spore trap samples. Factors such as initial training, type of microscope optics, visual acuity of the analyst, staining color, magnification, reading direction, etc., should be investigated.

Therefore, if low level spore trap results are to be used in a legal case, it would be prudent for the expert to have the spore traps read by more than one lab or, at a minimum, by a second microscopist and report the variation.

Based on these two research studies, here are some recommendadons for IEPS and analysis laboratories:

1. IEPs need to choose a lab for which they are confident in the reported results and to use a sampler that is appropriate for the environment being evaluated.

2. IEPs should do culturable sampling along with spore traps. They inherently have less variability in sampling analysis and are comparable to a large number of microbial standards worldwide.

3. Because of the variability in lab analysis results, IEPs should consider applying the need for a minimum difference between comparative samples as an acceptability criterion for PRV.

4. Laboratories should offer a “replicate” spore trap analysis service for legal cases. This service would use two microscopists to evaluate spore nap slides. The results would then be reported as a range. This would better quantify the variance associated with visual identihcation and give the IEP higher confidence in the data

5. Laboratories should consider establish ing a special “recounting” or “expanded” counting service. This service would involve counting a much larger portion of the slide. (Since April, one lab now makes this service available.) The special analysis results would be reported with a variance (e. g. 1:25 percent) between the counts for each genus.

6. Laboratories should notify clients if clusters of spores were present. In this study,
one lab reported this as potentially being the reason for such high variability. Clusters provide one of those time-consuming counting challenges. Also, clusters can be broken up in Anderson type samplers and result in higher culturable levels.

Table 1. Properties of the Different Spore Trap Samplers

Type of Sampler	Flow Rate (m3/min)	Cut off Diameter	Sample Air Volume
Air-O-Cell	15	2.6 μ	75 liters
Allergenco D	20	1.7 μ	100 liters
Cyclex D	15	1.0 μ	75 liters
Micro 5	5	0.8 μ	25 liters

TABLE 6. LOW CONCENTRATION SAMPLES - Total Spore Counts (s/m3)						
	AREA 1		Std. Dev./ Average	AREA 2		Std. De Avera
	Range	Average		Range	Average	
CYCLEX D	270-1580	**875**	69%	160-1350	**655**	61%
ALLERGENCO	352-586	507	15%	40-720	396	58%
MICRO 5	400-1400	616	61%	280-690	475	32%
AIR-O-CELL	84-1386	707	66%	66-1933	529	123%
AVERAGE		676	53%		534	68%

TABLE 7. MEDIUM CONCENTRATION SAMPLES - Total Spore Counts (s/m3)						
	AREA 1		Std. Dev./ Average	AREA 2		Std. De Avera
	Range	Average		Range	Average	
CYCLEX D	252-560	383	30%	80-1070	**547**	68%
ALLERGENCO	190-866	509	44%	252-589	416	38%
MICRO 5	120-880	534	46%	80-1120	437	79%
AIR-O-CELL	252-1094	**716**	46%	157-867	379	74%
AVERAGE		536	41%		445	65%