In Press at Mycologia, published on June 16, 2010 as doi:10.3852/10-017

Short title: Spore trap analyses

A multi-laboratory comparative study of spore trap analyses

L.D. Robertson[1]

Robert Brandys

Occupational and Environmental Health Consulting Services Inc., 635 Harding Road, Hinsdale, Illinois 60512, www.oehcs.com

Abstract: Fungal spore trap analyses currently are being marketed to the medical and environmental industries as a means of evaluating fungal bioaerosols. No studies comparing the results of these analyses have been conducted among laboratories providing these services. In the current study we compared the results from seven such laboratories with four different commercial spore trap cassettes with samples from four environmental conditions. The conditions included indoor air from a single location in a building under low, moderate and high agitation, and a sample from outside the same building. The means, ranges and standard deviations of total spore counts per cubic meter were respectively: low agitation indoor 514, 40–1933, 395; moderate agitation indoor 446, 80–1120, 290; high agitation indoor, 5154, 1510–15278, 3335; and outdoor 16012, 3700–28959, 6600. Results were similarly variable for the 27 spore categories that contribute to the total count. No consistent difference was observed in the precision of the kinds of spore traps. We concluded that spore trap analyses should be used with caution and should not be used as a sole method of assessing fungal spore populations and that standardized methods of analysis must be developed that include information about analytical precision of the sample data.

Key words: Air-O-Cell, Allergenco D, Cyclex-D, Micro 5Z

INTRODUCTION

Fungal spore trap analyses are being marketed to the medical and environmental industries as a means to evaluate fungal bioaerosols indoors and outdoors. The results of spore trap samples currently are used exclusively in investigations of indoor environments for "mold" to predict human exposure, to justify remedial recommendations and implementation, to evaluate quality control and post-remedial assessments of mold remediation projects, to monitor total fungal bioaerosols in indoor air quality (IAQ) management practices and to report mold levels in daily weather reports. Worldwide the direct and indirect financial impact of fungal spore trap analysis can be estimated in the millions of dollars. Despite the widespread use of commercial spore trap services, no studies have been published regarding the overall reliability of the fungal spore data. Clearly the reliability of spore trap data is required if results are to be used as an exclusive means of evaluating indoor and/or outdoor spore populations and concentrations and ultimately to evaluate the validity of exposure potential or remedial success. The purpose of this study was to evaluate the precision of spore trap analysis by current commercial laboratories and to provide knowledge on the overall reproducibility and subsequent reliability of spore trap data.

MATERIALS AND METHODS

Individual sampling cassettes were obtained from a laboratory participating in this study. Spore trap cassettes that were evaluated included the Zefon Air-O-Cell™ (Zefon International, St Petersburg, Florida) and Allergenco D™, Cyclex-D™ and Micro 5™ (Environmental Monitoring Systems Inc., Charleston, South Carolina). Cut diameters for Zefon Air-O-Cell™, Allergenco D™, Cyclex-D™ and Micro 5™ were respectively 2.6 µm, 1.7 µm, 1.0 µm and 0.8 µm. The cut diameter is the aerodynamic measure of airborne particle at which the collection or recovery efficiency drops to less than 50%. The aerodynamic measure is a function of the physical size, shape and density of the particle. The collection efficiency of the individual sampling cassette decreases with smaller diameter particles and increases with large particles with respect to the cut diameter.

The sampling was performed Aug 2006 in Chicago, Illinois, USA, in a condominium with a history of water leaks. Indoor conditions were 21 C with relative humidity (RH) 38%. Outside conditions were 20 C with RH 38%, less than 8 km/h wind and no precipitation. Sample cassettes and tubing were bundled in a circular design of approximately 6.5 cm diam to create a specific collection zone approximately 1.5 m from the floor/ground. All air inlet orifices/slits for the cassettes were assembled approximately 6.5 cm from each other in a rosette pattern and oriented so that the orifices faced away from the center of the bundle. Collections were made with the four sampling cassettes simultaneously under each of the four independent sampling conditions. Treatment 1 involved low air agitation samples collected indoors under normal ambient and quiescent conditions. Treatment 2 involved medium air agitation samples collected from the same environment with fans blowing on surfaces of the site. Treatment 3 involved high air agitation samples from the same environment with a high-pressure leaf blower on surfaces at the site. Treatment 4 involved samples collected outdoors under the conditions previously described.

Standard Gast vacuum pumps were calibrated individually at flow rates recommended by the manufacturers of the cassettes. The collection methods were those recommended by the manufacturer, resulting in 100l samples for Allergenco D and Micro5 samplers and 75 l samples for Air-O-Cell and Cyclex-D samplers.

We analyzed a total of 16 cassettes. The initial laboratory receiving the cassettes removed the microslides/filters from each cassette and individually affixed them to a standard microscope slide for standard light microscopic evaluation. The lab was allowed to use standard in-house operating procedures in the identification and enumeration of spores. Each laboratory reported that a single analyst deemed to be the most competent in performing spore trap analysis was assigned to study the slides. Each laboratory reported that 100% of the sample trace was evaluated. On completion of the microscopic examinations slides were labeled, placed in protective microscope slide cases and returned to the researchers, who then sent the slides to the next participating laboratory. This was repeated until all laboratories had analyzed the slides. Participating laboratories offered commercially available spore trap services and were recognized as analytically proficient by the American Industrial Hygiene Association's (Fairfax, Virginia) Environmental Microbiology Proficiency in Analytical Testing (EMPAT) program.

Laboratories prepared and sent analytical reports containing qualitative and quantitative data on fungal spores to the researchers. The laboratories developed a list of categories of fungal bioparticulates. Concentrations of fungal spores were reported as spores per cubic meter of air. Data from each laboratory were used to determine the mean, range and standard deviation.

RESULTS

Findings from the total spore count from the laboratories were highly variable, with ranges varying by more than an order of magnitude in three of the four sampling treatments (TABLE I). Results from the individual spore categories also were highly variable with the standard deviation frequently exceeding the mean.

In a comparison of the results from the four samplers, compiled data from all laboratories did not yield consistent results (TABLE II). No one sampler consistently demonstrated better precision than the others. Cyclex-D and Air-O-Cell each had the highest standard deviations for two of the samples, but Air-O-Cell also had the lowest standard deviation for one of the samples.

Laboratories reported that pronounced spore characteristics and the absence of occult debris were evident in samples collected outside, which improved identification. However, despite those observations, Treatment 4 (outdoors) continued to reveal substantial variability in results (TABLE III).

Laboratories also reported that the abundance of occult debris in Treatment 3 (indoor high air agitation) encumbered the analytical process and the observation of spore characteristics (TABLE IV). Treatment 3 results were highly variable; however some labs produced findings that exhibited a low standard deviation with respect to the mean, while other labs reported that samples were too overloaded with occult debris to appropriately analyze.

DISCUSSION

Analysis of a spore trap involves microscopy of the morphology of spores collected on the adhesive surface. Miquel, the father of aerobiology, was the first to characterize the presence of fungal spores in the atmosphere in 1789 (Miquel 1883). In 1882 Saccardo developed the first and primary system for classifying the imperfect fungi by spore color and form (Davis 1920). Later

the combined works of Persoon (Ainsworth 1968), Fries (Ainsworth et al 1971) and others expanded the basis for classifying spores of basidiomycota and ascomycota. Over the next century these systems, with ongoing contributions from other researchers, continued to provide the basis for the direct analysis and classification of fungal spores in aerosols. Hirst (1952) provided the first design and description of a volumetric spore trap in which a known quantity of air is drawn through a narrow slit and the airborne particles are lodged on an adhesive surface, thus establishing a means to qualify and quantify fungal bioaerosols.

Over the past four decades the evaluation of environmental fungal bioaerosols using spore trap designs has become a multimillion dollar industry. Numerous commercially available spore trap samplers currently are marketed and being used in the characterization of fungal bioaerosols indoors and outdoors. Commercial samplers with self-contained suction capacities and using standard microscope slides with an adhesive include the Burkard personal volumetric air sampler and the Allergenco MK-III (Environmental Monitoring Systems Inc., Charleston, South Carolina). More recent spore trap designs incorporate the modality of a cassette that is linked to an external suction device and include products such as the Zefon Air-O-Cell, Cyclex D, Allergenco-D and Micro-5.

Spore trap samples routinely are sent to commercial laboratories offering fungal identification. The analysis of the spore trap is based in the principles of the Saccardo/Fries/Persoon systems, except that current day systems have been expanded to include numerous other general and specific spore categories. The expanded spore category system is currently promoted and marketed by industry in training and proficiency testing (McCrone, Chicago, Illinois, Pan American Aerobiology Association, Amherst, Massachusetts; EMPAT); however the reliability of data reported in this expanded spore category system has not been

validated. For example current laboratories purport their ability to distinguish among the morphologically similar spores of *Alternaria, Pithomyces, Bipolaris, Dreschlera* and *Curvularia*. Inherent variation associated with spore age, degradation, morphological diversity and impact orientation on the retaining adhesive generally suggests that such analysis in the absence of concurrent live culture techniques increased analytical error. However no criterion of reliability has been established for these or other spore categories.

The absence of a reliable method to determine the actual bioaerosol concentrations in environmental settings precludes the ability to evaluate the accuracy of spore trap analytical methods. Therefore precision or the reproducibility of values obtained under similar conditions offers the only practical means by which the reliability of spore trap data can be determined. Laboratories participating in laboratory accreditation programs do monitor the laboratory precision of individual analytical processes (A^2LA, American Association for Laboratory Accreditation, Frederick, Maryland; EMLAP, Environmental Microbiology Laboratory Accreditation Program-American Industrial Hygiene Association. Fairfax, Virginia). Such programs develop in-house evaluations of analytical methods; however these evaluations do not apply to individual and discreet sampling results. Therefore an accredited laboratory that consistently misidentifies a spore would reveal a high precision for the analytical method even though the results had zero accuracy. While such in-house precision evaluations are useful in the laboratory's ability to maintain the reproducibility of the analytical technique, such analytical precision is not applicable or transferable to actual data collected in the field or documented in a report. Research purports to establish techniques for counting spores, sampling cassette recovery and other technical aspects of spore trap methodology (Marchand et al 2008, Godish and Godish 2008); however the value of this and other spore trap research remains in question without an

evaluation of analytical reproducibility. In addition current guidelines and recommendations for indoor concentrations of spores with spore trap methodology must rely on reproducible results (Brandys and Brandys 2010, Rao et al 1996)

Traditional applications of statistical evaluations can be applied to naturally occurring scientific phenomena. The environmental collection of spores with spore trap methods might represent a random collection practice, which in theory could be evaluated by traditional scientific and statistical practices. However it remains unclear whether the actual analytical process imposed on the collection, which uses the subjective skills and experience of a microscopy analyst, is capable of producing nonbiased information. Data obtained from spore trap analysis actually might represent the sophisticated opinion of the analyst instead of a true scientific measurement. Bias might be a factor at several areas within the analytical process. In this study participating laboratories elected to use the most experienced and skilled analyst. This represents a prejudice against other analysts at the same laboratories who perform these types of analysis day to day. In addition the specific skills and experience of each microscopy analysts are highly subjective, might fluctuate and/or be subject to distraction or misinterpretation, which also represents an inherent bias in the analytical process. We have evaluated spore trap results with the traditional statistical parameters of mean, range and standard deviation.

Data from this study demonstrated that commercial laboratories purporting to be proficient in the identification of spore traps differ largely on what is classified as a “total spore count”. Considerable differences were observed in reporting concentrations of individual fungal spores over the variety of sampling conditions in this study. Such variation among laboratories raises questions concerning the overall validity of spore trap methodology as a means to measure fungal bioaerosols. This study also revealed fundamental inconsistencies in the commercial

practice of spore trap analysis with respect to when a sample can actually be appropriately analyzed. No current laboratory guideline or standard exists with regard to when a sample has sufficient occult debris to be deemed overloaded and incapable of examination. These data further suggest that indoor environments having elevated concentrations of airborne dust and debris might affect analyses.

CONCLUSIONS

The intent of this study was to assess the ability of analytical laboratories to recognize both spore genera and number of fungal spores with various spore trap methods. The data demonstrated that the seven AIHA-accredited laboratories were not able to reliably perform these analyses. This type of analysis is fundamental for the use of spore traps within mold assessment, health and weather-related industries. Data revealed that only 75% of the accredited laboratories consistently identify *Cladosporium*, the most common mold in the environment. Furthermore *Aspergillus/Penicillium*-like spores, the most common mold category related to water intrusion, were identified by only 50% of the laboratories. This research reveals that precision of spore trap analyses, even among laboratories involved with analytical proficiency testing, lack precision and should be interpreted with caution. In our opinion the analysis of spore trap samples ultimately are subjective decisions based on the skill and experience of each microscopy analyst and the data might not be amenable to traditional statistical analysis of random environmental samples. We conclude that the results of spore trap analysis are highly variable and should not be used as a sole method for assessing fungal spore concentrations and populations until analytical precision can be demonstrated and documented with each analysis. We recommend:

1. Commercial laboratories offering spore trap analysis should immediately modify analytical processes so that individual precision is reported for each collection.

Analytical results should be reported with a variance (e.g. ± 25%) for total spore counts and individual spore categories. We further recommend use of culturable airborne sampling methods as an alternative and/or supplemental means to evaluate fungal bioaerosols.

2. Organizations/individuals providing training in the analysis of spore traps should seek standardization under an appropriate oversight organization. We suggest that the Mycological Society of America, Pan American Aerobiology Association or Indoor Environmental Standards Organization act as nonbiased organizations to provide appropriate oversight. Any course materials and training should be subject to appropriate oversight and review from independent sources having specific expertise in this area of mycology. Certification in analytical proficiency should address individual analysts in addition to laboratory practice and procedure.
3. Spore trap analysts performing commercial service immediately should seek to participate in programs to identify and improve analytical precision on a voluntary basis. Pan American Aerobiology Association currently has a voluntary program to assist in improving analytical precision. We suggest that the Mycological Society of America and the Indoor Environmental Standards Organization also provide intralaboratory precision evaluation and monitoring.

ACKNOWLEDGMENTS

We thank the laboratories participating in this study. While our results raise questions about the validity of current spore trap data, these laboratories assisted in the identification of deficiencies that have provided a clear focus on how the practice of spore trap science can be improved.

LITERATURE CITED

Ainsworth GC. 1968. Chapter 19: the number of Fungi. In: Ainsworth GC, Sussman AS, eds. The Fungi, an advanced treatise. Vol. III. New York: Academic Press. p 510.

———. 1971. Ainsworth & Bisby's dictionary of the fungi. Kew, Surrey, England: Commonwealth Mycological Institute. 663 p.

Brandys R, Brandys G. 2010. Worldwide exposure standards for mold and bacteria—historical and current perspectives. 8th ed. Hinsdale, Illinois: OEHCS Publications. 68 p.

Davis JJ. 1920. Pier Andrea Saccardo. Bot Gaz 70:156–157.

Godish D, Godish T. 2008. Total airborne mold particle sampling: evaluation of sample collection, preparation and counting procedures and collection devices. J Occup Environ Hyg 5:100–106.

Hirst JM. 1952. An automatic volumetric spore trap. Ann Appl Biol 39:257–265.

Marchand J, Cloutier Y, Pepin C, Drolet D. 2008. The effect of the number of counted traverses on the estimation of total spore count sampled on a non-cultivable slit impactor. J Environ Monit 10:1060–1063.

Miquel P. 1883. Les organismes vivants de l'atmosphère. Paris: Gauthier-Villars. 342 p.

Rao CY, Burge HA, Chang JC. 1996. Review of quantitative standards and guidelines for Fungi in indoor air. J Air Waste Manage Assoc 46:899–907.

FOOTNOTES

Submitted 21 Jan 2010; accepted for publication 25 May 2010.

[1]Corresponding author. E-mail: ldr@iecinc.net

TABLE I. Mean, range and standard deviation for spore counts/m^3 for indoor and outdoor environments

	Treatment 1 (low agitation)[a]			Treatment 2 (medium agitation)[a]			Treatment 3 (high agitation)[b]			Treatment 4 (outdoors)[a]		
	Mean	**Range**	**SD**	**Mean**	**Range**	**SD**	**Mean**	**Range**	**SD**	**Mean**	**Range**	**SD**
Total spore count	**514**	**40–1933**	**395**	**446**	**80–1120**	**290**	**5154**	**1510–15287**	**3335**	**16012**	**3700–28959**	**6660**
Alternaria	24	0–127	36	1.0	0–27	5	103	0–280	88	264	0–541	136
Arthrinium	1.1	0–30	6							1.4	0–25	5
Ascospores	34	0–240	49	40	0–160	45	185	0–560	153	2940	1067–6800	1260
Aspergillus/Penicillium–like	149	0–1453	299	104	0–560	148	1259	0–3720	1039	558	0–1970	527
Basidiospores	52	0–508	99	27	0–160	38	838	0–7220	1633	3021	0–11520	2972
Bipolaris/Dreschlera	0.8	0–13	3				37	0–573	134	10	0–120	29
Botrytis							2	0–27	6			
Cercospora							0.6	0–10	2	77	0–240	76
Chaetomium							0.6	0–10	2			
Cladosporium	127	0–600	138	251	0–880	196	2441	0–4480	1478	8627	1800–17075	3854
Curvularia	0.7	0–10	3							11	0–42	14
Clear brown	5	0–80	16	2	0–27	7	2	0–40	9	7	0–126	25
Colorless	0.5	0–13	2	0.5	0–13	2	50	0–750	178	13	0–213	42
Epicoccum	11	0–80	19				20	0–67	21	33	0–110	31
Fusarium										20	0–200	47
Nigrospora							17	0–80	22	25	0–370	70
Oidium/Peronospora										4	0–42	8
Paecilomyces										159	0–4450	841
Pithomyces	7	0–42	12	2	0–20	5	15	0–67	20	22	0–40	21
Rusts	5	0–40	10	2	0–13	4	3	0–13	6	3	0–40	9
Smuts/Myxomycetes/*Periconia*	57	0–340	77	11	0–53	17	143	0–320	102	186	0–675	209
Stachybotrys										0.5	0–13	2
Stemphylium										5	0–42	12
Trichoderma	18	0–360	71									
Torula							3	0–27	7	11	0–67	20
Ulocladium	4	0–40	12	0.7	0–10	3	8	0–120	28	1.0	0–27	5
Unclassified conidia	17	0–240	49	4	0–80	16	29	0–80	32	15	0–230	46

[a] n = 28 (seven laboratories and four collection methods).

[b] n = 18 (some labs reported overloads on Micro 5, Allergenco and Air–O–Cell collection methods).

TABLE II. Mean, range and standard deviation for total spore counts/m^3 for sampling methods indoors and outdoors

Sampling method	Sampling condition 1 (low agitation)[a]			Sampling condition 2 (medium agitation)[a]			Sampling condition 3 (high agitation)[b]			Sampling condition 4 (outdoors)[a]			Ave. mean	Ave. SD
	Mean	Range	Std dev	Mean	Range	Std dev	Mean	Range	Std dev	Mean	Range	Std dev		
Clyclex D	655	160–1350	402	553	80–1070	368	3876	1510–7240	2606	17047	3700–26010	7749	5533	2781
Allergenco	396	40–720	229	416	252–613	158	4679	1973–6986	2139	11806	6414–27649	7179	4324	2426
Micro 5	475	280–690	150	437	80–1120	348	5795	2840–8480	2334	18995	13254–28959	7072	6426	2476
Air-O-Cell	529	66–1933	651	379	157–867	280	6704	1573–15287	5965	16199	13960–19960	2385	5952	2320
Average	514		358	446		288	5263		3261	16012		6096		

[a] n = 28 (seven laboratories and four collection methods).

[b] Three laboratories reported overload.

TABLE III. Individual laboratory mean, range and standard deviation for total spore count (m^3) for Treatment 4 (outdoors)

Laboratory[a]	Mean	Range	Std dev
1	21941	13960–27863	6944
2	19406	11452–26010	6121
3	18321	6414–28949	9334
4	12787	8736–14594	2721
5	12888	10767–14440	1721
6	11295	3700–19960	6971
7	15445	9360–24460	6412

[a] n = four for each laboratory (one for each sampling cassette type).

TABLE IV. Individual laboratory mean, range and standard deviation for total spore count/m^3 for Treatment 3 (high agitation)

Laboratory	Mean	Range	Std dev
1[a]	4242	1610-6360	1965
2[a]	9183	5980-15287	4197
3[b]		Overload	
4[b]		Overload	
5[a]	6226	5500-7240	830
6[a]	2357	1573-3040	698
7[c]	2371	1510-3232	1218

[a] n = 4 (one per each sampling cassette type).

[b] Reported overload for all sampling methods.

[c] n = 2 Reported overload for Micro 5 and Air-O-Cell methods.