

**A COMPARISON OF SAMPLING METHODS FOR
DETECTING FUNGAL CONTAMINANTS IN CARPETS**

Joe C. Spurgeon
Daniel W. Bridge
Marshall E. Krotenberg

January, 2006

(

ABSTRACT

The purpose of the current study was to compare three carpet sampling methods based on micro-vacuum sampling using 25 mm filter cassettes. The three methods were closed-face, variable area (CFV), open-face, variable-area (OFV), and open-face, fixed-area (OFF). The comparisons were made using actual field samples rather than a controlled side by side comparison.

The 131 samples collected using the variable-area methods included 64 CFV samples and 65 OFV samples. The results for the two methods were reported on both a weight (cfu/g) and an area (cfu/100 cm²) basis. Results for the 55 fixed-area samples were only reported on an area basis.

The carpet samples were classified as either dry/clean or wet/contaminated. The CFV samples included 33 dry and 31 wet carpets; the OFV samples included 56 dry and 9 wet carpets; and the OFF samples included 26 dry and 29 wet carpets.

The sample results were stratified for analysis according to the following factors: (1) sampler configuration [closed-face v. open-face]; (2) sample area [variable area v. fixed area]; and (3) data reporting method [weight basis v. area basis].

The three sampling methods were more robust than anticipated. The three methods all performed similarly, and each method was capable of discriminating between the GM concentrations of dry/clean and wet/contaminated carpets.

The CFV and OFV methods were not statistically different based on the 95 % confidence interval (CI) on the GM when reported on either a weight or an area basis. The GM concentrations for dry and wet carpets were statistically different for both methods based on the CI on the GM. The OFV and OFF methods were not statistically different based on the CI on the GM.

The data evaluated in the current study were collected using three different sample collection methods, they were collected from projects in three states by various personnel, and the samples were cultured on different media. Even with these limitations, the estimated upper limits (95th percentile concentrations) for total fungi in dry/clean carpets were in a relatively narrow range. On a weight basis the range was 40,000 to 70,000 cfu/g; and on an area basis the range was 300 to 500 cfu/100 cm².

Key Words: carpet dust, micro-vacuum, filter cassette, carpet fungi, indoor mold

INTRODUCTION

Carpeting is a common flooring material in both residential and commercial structures. However, if subjected to water intrusion and then inadequately dried or maintained, carpets and pads can become a reservoir for fungal and other microbial contaminants. The contaminated carpeting can become a direct source of exposure for occupants due to the impact of footfalls, routine vacuuming, or other physical disturbances.⁽¹⁻⁵⁾

Various sampling methods have been reported for the collection of microbial contaminants in carpets, including standard vacuums equipped with collection bags, contact plates, and micro-vacuum methods using cassettes.⁽⁶⁻¹¹⁾ Although a standard method exists for the collection of lead dust in flooring, there is not a standard method for collecting microbial contaminants.⁽¹²⁾

There is a substantial database of carpet sample results available for comparison. But these samples were collected by various methods and under uncontrolled conditions. Therefore, the comparability of these data have not been determined.

The current study compares three micro-vacuum cassette sampling methods for the collection of carpet dust, with one method being similar to the standard method for collecting lead dust in carpets.⁽¹²⁾ The data were collected under actual field conditions, and reflect the various confounding factors associated with existing databases of similar results. The inclusion of these confounding factors in the comparison of sampling methods is representative of existing field data.

Background

Hodgson and Scott reported on 243 carpet samples collected in both problem and control buildings.⁽⁷⁾ The samples were collected using 37 mm cassettes containing 0.8 μ m polycarbonate filters. The results were reported as cfu/g of sample, but neither the airflow rate used to collect the samples nor the status of the cassette, closed-face or open-face, was reported.

The 19 samples collected in control buildings ranged from less than 400 cfu/g to 10^5 cfu/g, with a median concentration of about 23,000 cfu/g. The 224 samples collected in problem buildings ranged from less than 400 cfu/g to 10^8 cfu/g, with a median concentration of about 206,000 cfu/g.⁵

These authors concluded that total fungal concentrations in carpets greater than 10^5 cfu/g were likely to be associated with buildings contaminated with fungi, although concentration alone should not be the only factor affecting the classification of the carpet.

Uncontaminated houses were defined by Horner et al as those having less than 2 ft² of visible mold.⁽⁸⁾ One hundred carpet dust samples were collected from 50 such houses using a woven vacuum dust collection bag inserted in a vacuum cleaner hose. A minimum area of 1 m² was vacuumed, and the results were reported as cfu/g of dust. Four median concentrations of total fungi were reported; two for different culture media, winter concentrations, and summer concentrations. The median concentrations were in a relatively narrow range between 27,000 and 52,000 cfu/g of dust.

Bart, et al reported the range of initial fungal concentrations in carpet maintained at 80 °f and 80 % relative humidity in a chamber to be 186 cfu/100 cm² to 267 cfu/100 cm², with an average of 230 cfu/100 cm².⁽⁹⁾ The data were for four samples each of new carpet and used carpet that had been cleaned. The carpets were sampled using contact plates. The initial concentration range, roughly 190 cfu/100 cm² to 270 cfu/100 cm², might be suggestive of background concentrations for clean carpet reported on an area basis.

The average concentration in the study by Bart et al was reported to decrease from 230 cfu/100 cm² to about 6 cfu/100 cm², or 96 %, over an eight-week period. This result implies that the concentration of culturable fungal contaminants in carpets, once remediated and placed back in a normal environment, would be expected to decrease with time. This might be a consideration when evaluating results for post-remediation verification sampling.

Spurgeon used a 25 mm cassette to collect open-face, fixed-area samples on both dry and wet carpets.⁽¹⁰⁾ This method was similar to the standard method for lead dust in carpets, and those data have been included in the current study. The results were reported on an area basis as cfu/100 cm². The 95th percentile concentration for total fungi in dry carpets was reported as about 200 cfu/100 cm², and 300 cfu/100 cm² was suggested as a reasonable upper limit for total fungi in dry, clean carpets. This value was consistent with those derived from the report by Bart et al.⁽⁹⁾

The sampling method described by Hicks et al was essentially the same as the open-face, variable-area method described in the current study, except the samples were cultured on different media.⁽¹¹⁾ They used an open-face 25 mm cassette at an airflow rate of 25 lpm to collect carpet samples in 26 uncontaminated residences. The sample areas varied from 0.09 to 0.7 m² of carpet. The samples were collected from a high traffic area, a low traffic area, and from covered furniture or bedspreads.

Hicks et al reported several conclusions. First, it was concluded that a total fungal concentration in carpets greater than 10⁵ cfu/g was not indicative of fungal contamination. A value of 10⁶ cfu/g was suggested as being a more realistic upper limit for uncontaminated carpets.

Second, the authors concluded that the 10- to 40-fold variability in eight collocated carpet samples collected in a high traffic area was unexpected, and therefore (1) vacuum dust samples collected from immediately adjacent sections of carpet were not reliably reproducible, and (2) large sample sizes would be required in order to characterize the condition of the carpets in a building.

The authors suggested that one factor resulting in differences between the results in their study and those reported by other authors were differences in the sample collection methods. A third conclusion was that a standardized dust collection and analytical method should be used to compare results from different studies.

Purpose

Therefore, the lack of a standardized sampling method for microbial contaminants in carpets is an important issue to field investigators. The purpose of the current study was to compare three carpet sampling methods based on micro-vacuum sampling using 25 mm filter cassettes. The carpet samples collected using the three sampling methods were stratified for analysis according to the following factors:

- Sampler configuration [closed-face, open-face];
- Sample area [variable area, fixed area]; and
- Reporting basis [weight basis, area basis].

The primary purpose of the current study was to evaluate the effect of these three stratification factors on the reported data, with the objective of identifying a preferred sampling method. This included comparing the three sampling methods on their ability to classify carpets as either dry/clean or wet/contaminated.

The second objective was to compare the upper concentration limits of total culturable fungi that were associated with dry/clean carpets for each of the sampling methods. In addition, the conclusion of Hodgson and Scott that a concentration of total fungi greater than 10^5 cfu/g was indicative of fungal contamination was compared with the conclusion of Hicks et al that only concentrations greater than 10^6 cfu/g were indicative of fungal contamination.

The third objective was to compare the weight and area methods of reporting data. The relative advantages of these methods were discussed.

METHODS

The current study included a total of 189 carpet dust samples collected in both residential and commercial properties. The samples were collected using standard 3-piece 25 mm cassettes containing 0.8 μ m mixed cellulose ester (MCE) filters supported on cellulose pads. The results for the two variable-area sampling methods were reported on both a weight (cfu/g) and an area (cfu/100 cm²) basis. The results for the fixed-area sampling method were only reported on an area (cfu/100 cm²) basis.

Variable-area Samples

The samples were collected by either marking an area of carpet with tape or using a template, then brushing the sampler across the surface of the carpet in a horizontal and then vertical pattern. The airflow rate used to collect both types of variable-area samples was 20 lpm, calibrated using a 60 mm rotameter with a range of 0-30 lpm.

Although the sample area was measured for each sample, the carpet area that was sampled varied between samples, ranging from 929 cm² to 25,083 cm². Since the sample areas were known, the results for the variable-area samples have also been reported on an area basis as cfu/100 cm². The limit of detection (LOD) was equal to or less than 0.1 cfu/100 cm².

The samples were analyzed by an AIHA (EMLAP) accredited laboratory after culturing on potato dextrose agar (PDA). The results were reported as colony forming units per gram of dust (cfu/g); and both the sample weight and analyzed weight (weight of the sample that was actually analyzed) were reported for each sample.

The variable-area samples were collected during 25 residential and commercial investigations that were conducted in Arizona, Nevada, and Texas; and were collected by at least six different field personnel. The 104 residential samples included 56 samples collected from 16 houses, 25 samples from an apartment complex, eight samples from a condominium complex, and five samples from a mobile home. The 25 commercial samples included 12 samples from a hotel, 10 samples from a church, and three samples from an office building.

The closed-face, variable-area samples included 12 controls. Four of the controls were collected from a non-complaint motel and eight from a large apartment complex. The carpets classified as dry/clean were located in complaint buildings, but were not visibly stained and did not show signs of having been directly impacted by water intrusion. The carpets classified as water damaged had visible evidence that they had been directly affected by a water intrusion.

The 64 closed-face, variable-area (CFV) carpet samples included 12 controls, 21 dry carpets, and 31 wet carpets. The controls were combined with the dry carpets, resulting in a sample size of 33 for the dry carpets.

The variable-area samples were collected using two sampling methods. The first sample collection method used closed-face cassettes with a beveled tube attached to the inlet. The samples were collected by brushing the beveled tube across a measured area of carpet. Although the area of carpet was measured for each sample, the actual sample area varied between samples. These were referred to as closed-face, variable-area (CFV) samples.

The second sampling method used open-face cassettes to collect samples from measured areas of carpet. An open-face cassette was brushed across the surface of the carpet within the measured sample area. The sampled area of carpet was measured for each sample, but the actual area that was sampled varied between samples. The 65 open-face, variable-area (OFV) carpet samples included 56 dry carpets and 9 wet carpets.

Fixed-area Samples

The third method used open-face cassettes to sample a fixed area of carpet for each sample.⁽¹⁰⁾ The samples were collected by firmly holding the open cassette in a static position against the carpet for five seconds, then repeating this procedure for a total of 20 locations on the carpet. These were referred to as open-face, fixed-area (OFF) samples.

The 55 samples were collected in 31 residential condominium units. Eleven of the fixed-area carpet samples were classified as controls, 15 as dry/clean, and six as water damaged. An additional 23 carpets, those in proximity to potential sources of leaks but not visually confirmed as damaged, were classified as potentially water damaged. The potentially wet carpets were combined with the wet carpets, resulting in a total of 29 carpets.

The airflow rate was calibrated to 10 lpm using a 60-mm rotameter. The area sampled was 98 cm², with a total contact time of 100 seconds. The fixed-area samples were cultured on malt extract agar (MEA) and results were reported on an area basis (cfu/100 cm²). The relatively small sample area resulted in a limit of detection (LOD) of 100 cfu/100 cm², compared to a LOD of 0.1 cfu/100 cm² or less for the variable-area samples.

The filter sample was analyzed by removing the filter from the cassette, placing it in a volume of sterile water, and vortexing. The solution was brought to a known volume, and an aliquot was pipetted onto a culture plate. The fraction of spores released from the filter was not reported by the laboratory. However, it was assumed that the portion retained on the filter was a characteristic of the method, and was expected to be relatively constant between samples.

RESULTS

The data obtained for dry/clean carpets are summarized in Table I for the three sampling methods. The data are reported on both a weight basis and an area basis. The parameters included in Table I are the sample size (N); concentration range (min, max); the geometric mean (GM) concentration; the 95 % confidence interval on the GM (CI), bounded by the lower and upper confidence levels (LCL, UCL); geometric standard deviation (GSD); and the 16th and 95th percentile concentrations (C₁₆, C₉₅).

Table I. Comparison of data for dry carpets collected using closed-face and open-face cassettes and reported on both a weight and an area basis.

PARAMETERS	CFV (cfu/g)	OFV (cfu/g)	CFV (cfu/100 cm ²)	OFV (cfu/100 cm ²)	OFF (cfu/100 cm ²)
Number	33	56	33	56	26
Minimum	600	180	1	0.5	50
Maximum	96,000	120,000	1,000	860	700
LCL_GM	4,400	7,100	15	25	52
GM	6,500	9,700	25	36	66
UCL_GM	9,500	13,000	42	50	84
GSD	6.5	3.2	10.4	4.0	1.9
C ₁₆	2,100	3,000	6	9	35
C ₉₅	40,000	67,000	290	360	190

Sampler configuration [closed-face v. open-face]: The concentration ranges and 95 % confidence interval (CI) on the GM concentrations were similar for the CFV and OFV methods when reported on either a weight or an area basis. The concentration ranges for dry carpets obtained using the closed-face and open-face cassettes were equivalent.

The GSD for the closed-face methods (6.5, 10.4) in Table I were higher than for the open-face methods (1.9, 3.2, 4.0). The results for the open-face cassette were less variable, offering a potential advantage when attempting to discriminate between clean and contaminated carpets based on small sample sizes. However, a lower GSD may also indicate a less efficient sampler.

The OFF method was the least variable. However, the low GSD for the OFF method was influenced by the 20 out of 26 samples that were censored due to a relatively high LOD of 100 cfu/100 cm². A value of LOD/2 (50 cfu/100 cm²) was substituted for the censored data.

Sample area [variable area v. fixed area]: The OFV and OFF data in Table I, considering the high proportion of censored data for the OFF method, were essentially equivalent. The smaller sample areas associated with the OFF method did not result in greater variability in the data. Therefore, the time required to sample 900 to 25,000 cm² using the OFV method did not provide any additional benefit compared to the time required to sample 98 cm² using the OFF method.

The data obtained for wet/contaminated carpets are summarized in Table II for the three sampling methods. The data are reported on both a weight basis and an area basis.

Table II. Comparison of data for wet carpets collected using closed-face and open-face cassettes and reported on both a weight and an area basis.

PARAMETERS	CFV (cfu/g)	OFV (cfu/g)	CFV (cfu/100 cm ²)	OFV (cfu/100 cm ²)	OFF (cfu/100 cm ²)
Number	31	9	31	9	29
Minimum	9,400	210,000	510	2,000	200
Maximum	580,000,000	2,300,000	1,900,000	14,000	110,000
LCL_GM	480,000	530,000	6,100	2,500	1,600
GM	1,200,000	970,000	14,000	4,30	3,100
UCL_GM	3,000,000	1,800,000	32,000	7,200	6,200
GSD	13.5	2.3	10.4	2.1	6.4
C ₁₆	89,000	430,000	1,400	2,100	490
C ₉₅	87,000,000	3,800,000	660,000	14,000	66,000

Sampler configuration [closed-face v. open-face]: The CI on the GM concentrations were similar for the CFV and OFV methods when reported on a weight basis in Table II. The variations in the C₁₆ and C₉₅ concentrations reported on a weight basis for the two methods were attributed to the small sample size for the OFV method.

When reported on an area basis, the concentration ranges for the CFV and OFV methods in Table II were not similar. The OFV data were in a relatively narrow range compared to the CFV data. However, the CI for these concentration distributions indicated the GM concentrations were not statistically different. Therefore, this variation may have been the result of the small sample size for the wet carpets.

The GSD for the closed-face methods (10.4, 13.5) in Table II were higher than for the open-face methods (2.1, 2.3, 6.4). The results for the open-face methods were less variable for wet carpets, as they were for the dry carpets in Table I.

Sample area [variable area v. fixed area]: The CI for the OFV and OFF data in Table II indicated the GM concentrations for the two methods were not statistically different. However, a wider range of concentrations was reported for the OFF method compared to the OFV method.

Reporting basis [weight basis v. area basis]: The following discussion refers to a comparison of the data for dry/clean carpets contained in Table I with the data for wet/contaminated carpets contained in Table II.

The differences in the GM concentrations for the CFV data reported on a weight basis were statistically different based on the CI. The UCL for dry carpets was 9,500 cfu/g, and the LCL for wet carpets was 480,000 cfu/g. In addition, the C_{16} for wet carpets was 89,000 cfu/g and the C_{95} for dry carpets was 40,000 cfu/g (ratio = 2.2). The results were similar for the OFV method when reported on a weight basis. Therefore, the CFV and the OFV methods were both able to discriminate between the GM concentrations obtained for dry/clean and wet/contaminated carpets when reported on a weight basis.

The differences in the GM concentrations for the CFV data reported on an area basis were also statistically different based on the CI. The UCL for dry carpets was 42 cfu/100 cm², and the LCL for wet carpets was 6,100 cfu/100 cm². In addition, the C_{16} for wet carpets was 1,400 cfu/100 cm² and the C_{95} for dry carpets was 290 cfu/100 cm² (ratio = 4.8). The results were similar for the OFV method when reported on an area basis. Therefore, the CFV and the OFV methods were both able to discriminate between the GM concentrations obtained for dry/clean and wet/contaminated carpets when reported on an area basis.

Reporting methods and carpet classification: The relative abilities of the OFV and OFF methods to discriminate between dry/clean and wet/contaminated carpets were compared based on the dry/clean data in Table I and the wet/contaminated data in Table II.

The differences in the GM concentrations for the OFV data reported on an area basis were statistically different based on the CI. The UCL for dry carpets was 50 cfu/100 cm², and the LCL for wet carpets was 2,500 cfu/100 cm². In addition, the C_{16} for wet carpets was 2,100 cfu/100 cm² and the C_{95} for dry carpets was 360 cfu/100 cm² (ratio = 5.8).

The differences in the GM concentrations for the OFF data reported on an area basis were also statistically different based on the CI. The UCL for dry carpets was 84 cfu/100 cm², and the LCL for wet carpets was 1,600 cfu/100 cm². In addition, the C_{16} for wet carpets was 490 cfu/100 cm² and the C_{95} for dry carpets was 190 cfu/100 cm² (ratio = 2.6).

Therefore, the OFV and the OFF methods were both able to discriminate between the GM concentrations obtained for dry/clean and wet/contaminated carpets when reported on an area basis.

DISCUSSION

Stratification Factors

Two micro-vacuum carpet sampling methods, closed-face and open-face cassettes, have been compared. The first objective of the study was to compare the three sampling methods based on sampler configuration, area sampled, and data reporting method.

The first method involved brushing the inlet of the cassette, either closed-face or open-face, over the surface of the carpet. This method would be more likely to emphasize the collection of surface rather than deep-seated contaminants.

In addition, the possibility exists for some areas of the carpet to be sampled multiple times, and some areas not at all. Therefore, there is the potential for the actual area sampled to vary from the defined sample area. Finally, depending on how the cassette is held, the suction force applied to the carpet within the sampled area may vary during the sampling period.

The second method was similar to the ASTM method for collecting carpet dust for lead analysis.⁽¹²⁾ An open-face cassette was calibrated to a known airflow, which was representative of an applied negative pressure, and held firmly against one spot on the carpet for a known period of time (5 seconds). The area of an open 25 mm cassette is 4.9 cm². The area sampled is that area multiplied by the number of spots included in the sample.

With this method, the area sampled was known and reproducible, the negative pressure applied to each area of the carpet was constant, and the contact time was closely estimated. In addition, this method should be more likely to collect both surface and deep-seated contaminants. Based on these considerations, the OFF method was expected to be the preferred method based on a comparison of field data.

However, the data reported for the OFV and OFF methods were similar. Both methods were able to distinguish between the GM concentrations for dry/clean and wet/contaminated carpets contained in Table I and Table II..

One advantage of the OFF method compared to the OFV method was the apparent lack of benefit from the additional time required to sample 900 to 25,000 cm² using the OFV method compared to sampling 98 cm² with the OFF method. However, one limitation of this study is that these conclusions were not based on side by side sampling, but on a comparison of the two concentration distributions.

An apparent source of error was the relatively high LOD of 100 cfu/100 cm² for the OFF method. Twenty of the 26 samples (77 %) for dry/clean carpets collected using the OFF method were censored. However, in comparison, about 80 % of the samples collected using the OFV method were less than 100 cfu/100 cm². Therefore, the two distributions were similar, and the high percentage of censored data for the dry carpets sampled using the OFF method were not expected to affect the conclusions. The higher LOD associated with the OFF method was acceptable because the upper limit for dry/clean carpets exceeded the LOD of the method.

In comparing the CFV and OFV methods, the two concentration distributions for dry carpets in Table I were not statistically different based on a comparison of the CI on the GM. In addition, the two concentration distributions for wet carpets in Table II were not statistically different based on a comparison of the CI on the GM for the CFV and OFV methods. Therefore, essentially no difference in the closed-face and open-face sampling methods was detected for either dry or wet carpets.

Weight Basis v. Area Basis

Interpretation of the results for dry, clean carpets were similar when reported on either a weight or an area basis. However, there has been a trend towards reporting microbial concentrations in settled dust on an area basis.^(4, 5) Reporting results on an area basis may offer several advantages. For example, a moderate positive correlation between respiratory symptoms and fungal concentrations in settled dust was found when the results were reported on an area basis, but a negative correlation was obtained when reported on a weight basis.⁽³⁾

Second, if the laboratory reported the analyzed weight, but not the sample weight, the fungal concentration in the dust is known, but the fungal loading on the carpet can not be estimated. For example, it would be typical for a laboratory to report a result as 100,000 cfu/g based on the analysis of 0.1 gram of the submitted sample. This information is not sufficient to allow the total fungal loading on the carpet to be estimated unless the total amount of dust in the carpet was known.

If the sample weight were also reported, and the sample area was known, then the total fungal loading per unit area of carpet could be estimated ($\text{cfu/g} \times \text{g/cm}^2 = \text{cfu/cm}^2$). Reporting the results on an area basis provides the option of considering the relative impacts of a 1 m² area rug and a 10 m² installed carpet on the indoor environment.

Third, reporting on an area basis may be preferred if post-remediation verification sampling is anticipated. One can imagine a situation in which carpet dust contains 100,000 cfu/g of total fungi. Prior to cleaning, 100 cm² of carpet has to be sampled to collect a gram of dust. Post-cleaning, the dust still contains 100,000 cfu/g, but 1,000 cm² of carpet has to be sampled to collect a gram of dust. Intuitively, the carpet is cleaner, but reporting the results on a weight basis would not reflect the reduction in contaminant concentration that would be obvious if reported on an area basis.

Finally, the good agreement in this study between results reported on an area and weight basis was achieved because the laboratory not only reported the weight of sample that was actually analyzed, but also the total weight of the sample. This allowed the concentration to be calculated as cfu/sample ($\text{cfu/g} \times \text{g/sample} = \text{cfu/sample}$), which could then be converted to an area basis ($\text{cfu/sample} / \text{area sampled} = \text{cfu/unit area}$).

Variability

The higher GSD values reported for the CFV method compared to the OFV method, reflective of the restricted concentration ranges obtained with the OFV methods, suggested that the CFV method may have been a more efficient sampling method. The higher GSD value indicated a greater range in the data, resulting in the potential to detect smaller variations in the data. However, potential differences due to variations in the analytical procedures that were applied to the two methods were not explored.

Hicks et al also concluded that the 10- to 40-fold variability in eight collocated carpet samples collected in high traffic area was unexpected.⁽¹¹⁾ Therefore vacuum dust samples collected from immediately adjacent sections of carpet were not reliably reproducible, and large sample sizes would be required in order to characterize the condition of the carpets in a building. However, approximately a 40-fold range

of concentrations is necessary to produce a GSD of 2.7, which was the GSD reported for the distribution of concentrations in the high traffic areas. This degree of variability is typical of many concentration distributions for environmental samples.

The conclusion of the current study was that any of the three sampling methods that were described were able to distinguish between the GM concentrations for dry/clean and wet/contaminated carpets. This implies that a sufficient number of samples have been collected to calculate the GM concentration. However, it is not unusual for a hygienist to only collect one sample from a carpet. Therefore, as it relates to the typical field investigation, this conclusion is not always applicable. A decision criterion based on the results for a single sample would also be helpful.

Rationale for Numerical Guidelines

The objective of collecting carpet dust samples is to aid in the decision-making process. Carpet samples are collected and submitted to a laboratory in hopes of receiving a sample result, generally expressed as a numerical value.

The hygienist is generally tasked to interpret the laboratory results, deciding if the sampled carpet is clean or contaminated. Since consensus guidelines on the acceptable level of fungal contamination in carpets are not generally available, this decision is based on professional judgment; and that judgment is ultimately based on the experience obtained interpreting previous sample results.

Numerical guidelines may or may not be in a written form. However, the hygienist must rely upon numerical guidelines from some source to interpret the laboratory report and then classify the sampled carpet as clean or contaminated.

Numerical guidelines for classifying the condition of a carpet may be useful under at least three conditions. First, when a common environmental fungus such as *Cladosporium* spp. is the amplified fungus. The commonality of *Cladosporium* in the environment often makes it difficult to obtain a consensus among hygienists that the carpet is contaminated and should be remediated. A numerical guideline at least offers a reference point for evaluating amplification of even common environmental fungi.

Second, only a limited number of samples may be collected from the subject carpet, especially in residential investigations. The small sample size, frequently limited to just one sample, generally precludes the use of a statistical analysis in those investigations. Under those conditions numerical guidelines, written or unwritten, may be the only option for interpreting the sample results.

Third, numerical guidelines may be useful when remediated carpets have to be either accepted during post-remediation verification sampling, and control samples are not available for comparison. This is not an unusual situation, especially in residential investigations. However, it is emphasized that numerical guidelines are nothing more than the beginning point for the application of professional judgment.

Weight-Based Numerical Guidelines

Hodgson and Scott reported the median concentration of total fungi for carpets in control buildings as 23,000 cfu/g.⁽⁷⁾ The median concentration in problem buildings was reported to be 206,000 cfu/g. In addition, these authors proposed an upper limit of 10^5 cfu/g for carpets in control buildings. Horner reported that the median concentration in clean buildings ranged between 27,000 cfu/g and 52,000 cfu/g for total fungi. The values for control areas were similar for these two studies.

However, Hicks reported the GM concentration of total fungi for clean buildings to be 125,000 cfu/g to 170,000 cfu/g, with an LCL on the GM of 85,000 cfu/g and a UCL of 257,000 cfu/g.⁽¹¹⁾ These values were more representative of the median concentration reported by Hodgson and Scott for problem buildings rather than their control buildings.

The second objective was to evaluate the conclusion of Hodgson and Scott that a concentration of total fungi greater than 10^5 cfu/g was indicative of fungal contamination.⁽⁷⁾ This assertion was compared with the conclusion of Hicks et al that only concentrations greater than 10^6 cfu/g were indicative of fungal contamination.⁽¹¹⁾

In the current study, the 95th percentile concentration for dry carpets sampled using the CFV method was approximately 40,000 cfu/g. In comparison, the 10th percentile concentration for wet carpets sampled using this method was also about 40,000 cfu/g. Second, the 95th percentile concentration in this study for dry carpets sampled using the OFV method was approximately 67,000 cfu/g. These data suggested an upper limit for dry/clean carpet that was actually less than 10^5 cfu/g.

Therefore, it was concluded that the demarcation between the dry and wet carpets included in this study was in the range of 40,000 cfu/g to 70,000 cfu/g, depending upon the sampling method. These results were even lower than the upper limit of 10^5 cfu/g proposed by Hodgson and Scott, and did not support the limit of 10^6 cfu/g proposed by Hicks et al.

Area-Based Numerical Guidelines

It was assumed the range of concentrations associated with clean carpets would be limited, while the range associated with contaminated carpets would essentially be unbounded. Therefore, the emphasis was placed on assessing the range of total fungal concentrations typically associated with dry/clean carpet rather than wet/contaminated carpet.

The C_{95} for dry/clean carpets for the OFV method in Table I was 360 cfu/100 cm², and the 95 % UCL on C_{95} was 590 cfu/100 cm². The corresponding values for the OFF method were 190 cfu/100 cm² and 240 cfu/100 cm². Because of the high percentage of censored OFF data, the OFV data were assumed to be more representative of the actual concentration distribution. However, the sample size (9) for the OFV method was limited.

Therefore, the OFV and OFF data in Table I were combined, resulting in an uncensored concentration distribution for 82 dry/clean carpets. The 95th percentile concentration of total fungi for the 82 dry carpets was 330 cfu/100 cm², with a 95 % UCL on the C₉₅ of 520 cfu/100 cm².

The highly censored data reported by Spurgeon probably resulted in a low estimate for the single-sample upper limit for dry/clean carpet, which was a concentration of 300 cfu/100 cm².⁽¹⁰⁾ The uncensored data for the 82 dry/clean carpets suggests a range of 300 cfu/100 cm² to 500 cfu/100 cm².

SUMMARY

It should be emphasized that a lack of discussion concerning the impact of indicator species versus common environmental fungi on the interpretation of the sample results does not imply a lack of importance of those issues. The purpose of this study was the comparison of sampling methods, and other important factors affecting data interpretation were not addressed..

The three sampling methods described in this study were more robust than anticipated. The CFV, OFV and OFF methods all performed similarly, and each method was capable of discriminating between the GM concentrations of dry/clean and wet/contaminated carpets.

The data evaluated in the current study were collected using three different sample collection methods, and the samples were cultured on different media. Even with these limitations, the estimated upper limits for total fungi in dry/clean carpets were in a relatively narrow range. On a weight basis the range was 40,000 to 70,000 cfu/g; and on an area basis the range was 300 to 500 cfu/100 cm².

The relatively narrow concentration ranges for the upper limits for dry/clean carpets suggest that it may be feasible to develop numerical guidelines for fungal concentrations in dry, clean carpets based on a standardized sampling method and sampling protocol.

The GM concentrations for both closed-face and open-face cassettes, when used to sample variable areas, were not statistically different based on the CI on the GM when reported on either a weight or an area basis. The closed-face and open-face cassettes produced comparable results.

The GM concentrations for dry and wet carpets, obtained using both closed-face and open-face cassettes, were statistically different based on the CI on the GM.

The GM concentrations obtained with open-face cassettes using the variable area and fixed area methods were not statistically different based on the CI on the GM.

The upper limit for total fungi in dry/clean carpets was estimated to be 40,000 cfu/g to 70,000 cfu/g; and of 300 cfu/100 cm² to 500 cfu/100 cm².

REFERENCES

1. Beguin, H., N. Nolard: Prevalence of fungi in carpeted floor environment: Analysis of dust samples from living rooms, bedrooms, offices and school classrooms. *Aerobiologia*, 12:113-120 (1996).
2. Wickens, K., J. Doves, R. Seibers, P. Titzharris, J. Wouters, G. Dockes, K. Mason, M. Hearfield, M. Cunningham, J. Crane: Determinants of endotoxin levels in carpets in New Zealand homes. *Indoor Air*. 13(2):128-135 (2003).
3. Dharmage, S., M. Bailey, J. Raven, T. Mitakakis, A. Cheng, D. Guest, J. Rolland, A. Forbes, F. Thein, M. Abramson, E. H. Walters: Current indoor allergen levels of fungi and cats, but not house dust mites, influence allergy and asthma in adults with high dust mite exposure. *Am. J. Respir. Crit. Care Med.* 164:65-71 (2001).
4. Rao, C. Y., J. M. Cox-Ganser: Associations between respiratory health effects and allergens, fungi and endotoxin in settled dust: Relevant units of potential exposure. *Am J Respir Crit Care Med*, 167(7):A501 (2003).
5. Doves, J., A. Zuidhof, G. Dockes, S. van der Zee, I. Wouters, H. M. Boezen, B. Brunekreff: (1-3)- β -D-glucan and endotoxin in house dust and peak flow variability in children. *Am. J. Resp. Crit. Care Med.* 162:1348-1354 (2000).
6. Chew, G.L., Rodgers, C., Burge, H.A., Muilenberg, M.L., Gold, D.R., Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics; *Allergy*, 58:13-20 (2003).
7. Hodgson, M., Scott, R.J., Prevalence of fungi in carpet dust samples, *Bioaerosols, Fungi and Mycotoxins: Health Effects, Assessment, Prevention and Control*, pp. 268-74, E. Johannig (ed.), Albany, NY; Boyd Printing Co., 1999.
8. Horner, E.W., Worthan, A.G., Morey, P.R., Air- and Dustborne Mycoflora in Houses Free of Water Damage and Fungal Growth, *Appl. & Environ. Microbiol.*, 70(11):6394-6400, (2004).
9. Bart, M. A., K. Foarde, C. Mitchell, K. Bolden, C. Walton, R. Adama: Final Report of the Hydrolab Project 2001: Flooring, Humidity and Mold Growth. Carpet and Rug Institute, Dalton, GA; Feb. 20, 2002.
- 10 Spurgeon, J. C.: A sampling method for comparing fungal concentrations in carpets. *AIHAJ* 64:842-845 (2003).
11. Hicks, J.B., Lu, E.T., de Guzman, R., Weingart, M., Fungal types and concentrations from settled dust in normal residences, *JOEH*, 2:481-92, (2005).
12. American Society for Testing and Materials (ASTM): *Standard Practice for Collection of Surface Dust by Air Sampling Pump Vacuum Technique for Subsequent Lead Determination*. (ASTM Standard E 1973-99). ASTM, West Conshohocken, PA.; 1999.