A Sampling Protocol for Detecting Low Concentrations of Airborne Fungi in Highly Filtered Hospital Air Using the Bi-Air Filter Cassette

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INTRODUCTION

Sampling Protocol

This article describes a sampling protocol for the detection of airborne *Aspergillus/ Penicillium* (*Asp/Pen*) like fungal spores in highly filtered hospital air. The protocol is applicable to the detection of airborne fungal spores in critical care areas of hospitals, clean rooms, or other environments in which the expected concentration of fungal spores is very low. The protocol has been used for baseline sampling, incident response, and post-remediation verification sampling. Duplicate sample traces were collected on mixed cellulose ester (MCE) filter media using the Bi-Air (BA) filter cassette. One sample trace was analyzed by microscopy for the presence of *Asp/Pen* like fungal spores. If *Asp/Pen* like spores were detected, and a patient risk assessment was warranted, then the duplicate sample trace was submitted for analysis by quantitative polymerase chain reaction (QPCR); which was used to identify the spores to species.

The described sampling method allows a rapid initial exposure assessment to be performed based on the microscopic analysis of total fungal spores, plus the ability to rapidly perform a risk assessment using QPCR methods. This sampling protocol provides several advantages for performing an exposure assessment in highly filtered air: (1) microscopy allows an immediate exposure assessment to be performed; (2) sample volumes routinely exceeding 1,000 liters provide adequate sensitivity; (3) sampling times of 8 hours or more are easily achieved; (4) the number of samples to achieve a given LOD is minimized; (5) spores/m^3 is a more conservative assessment criterion than cfu/m^3 since each spore is count; and (6) QPCR analysis allows a rapid patient risk assessment to be performed when required. Finally, QPCR analysis, which is relatively expensive, is only required if the initial sample is positive for *Asp/Pen* like spores.

Exposure Assessments

Relatively low concentrations of infectious fungal spores in highly filtered air have been associated with the occurrence of infection in healthcare facilities. The document

Guidelines for Environmental Infection Control in Health-Care Facilities (HICPAC) states “aspergillosis cases have occurred when fungal spore concentrations in Protective Environment (highly filtered) air ranged as low as 0.9 ... colony forming units per cubic meter of air (cfu/m^3)”. Although anecdotal in nature, this concentration could be assumed to represent an upper limit to the acceptable airborne concentration of infectious fungi in critical areas when performing a risk assessment. This implied limit has several implications regarding the suitability of a sampling protocol for performing exposure assessments. It places constraints on the minimum sample volume, the sample collection time, the sample media, and the method of sample analysis.

The collection of airborne samples in healthcare facilities may have two broad objectives. The first is infection control, which may be characterized in industrial hygiene terms as a patient risk assessment. The objective of risk assessment sampling is to detect and identify airborne fungi to both genus and species, which then allows Infection Control personnel to assess the need for implementing risk management procedures. However, it may be beneficial to precede risk assessment sampling with an exposure assessment, which has the objective of determining if a potential exposure exists. The logic is that if there is no potential for exposure, then a risk assessment is not necessary. With reference to healthcare facilities, for example, it would only be necessary to perform a risk assessment (culture a sample for *Aspergillus fumigatus*) if the exposure assessment demonstrated that *Asp/Pen* like spores were present in the sampled environment.

Culturable Samples

Typically, the protocols for collecting airborne fungal samples in healthcare facilities have emphasized the collection of culturable samples, with the focus on risk assessment. However, this methodology has several limitations for performing exposure assessments, including short sampling times [high variability], a high limit of detection (LOD) [low sensitivity], a delayed response time, the detection of only culturable spores rather than total spores, and a high cost due to the need for the collection of multiple samples using multiple media at each sampling location.

First, a limit of detection (LOD) of 0.8 cfu/m^3, just below the implied concentration limit of 0.9 cfu/m^3, requires a minimum sample volume of 1,250 liters; although the ability to detect this concentration with 95 % confidence may require a sample volume as large as 2,500 liters. As an example, collecting a total sample volume of 1,250 liters using a Graesby-Andersen N6 Bioaerosol Sampler operating at an airflow rate of 28.3 lpm and a sample collection time of five minutes would require nine samples to be collected at each sampling location and for each culture media. The same number of samples would be required at each sample location to achieve a LOD of 0.8 spores/m^3 if 10-minute samples were collected using a slit impaction cassette operating at 15 lpm.

Second, the sample results should have as small a variance as possible in order to minimize both false positives and false negatives. Since disturbing patient populations is difficult, and may itself increase patient risk, the ability to interpret the sample results is an important characteristic of the sampling protocol. In general, when estimating the

average concentration, accuracy is associated with sample time and precision is associated with sample volume. Since long-term Time-Weighted Average (TWA) samples provide a better estimate of the average concentration present in a particular space, and typically have less variability than short-term "grab" samples, a sampling method that collects TWA samples would be preferred.

Third, the sample media affects the integrity of the sample, and also determines how the sample can be analyzed. For example, some slit impaction samplers are not efficient at collecting fungal spores with an aerodynamic diameter less than 2.7 um. Since *A. fumigatus* has a diameter of about 2 um, this spore may not be detected by slit impaction samplers when present at the very low concentrations typical in highly filtered air. In addition, the adhesive media typically used in slit impaction samplers does not lend itself to analysis by methods other than microscopy. However, filter media are commonly used to collect airborne fungal spores when performing exposure assessments. Filter cassettes are easy to use and are suitable for the collection of long-term TWA samples. Filter media such as MCE may be examined by microscopy, cultured on multiple culture media following sample collection, or analyzed by newer methods such as quantitative polymerase chain reaction (QPCR).

Fourth, the limitations of culturing as a method of analysis has been discussed. Vesper & Vesper have suggested QPCR as an alternative to culturing for the rapid analysis of spores. In the project they describe, a sample volume of 630 liters was collected on filters over a three-hour period at an airflow rate of 3 liters per minute (lpm), resulting in a LOD of 1.6 spores/m^3.

Purpose

This article describes a sampling protocol suitable for baseline sampling, incident response, and post-remediation verification sampling. Its application to the detection of airborne fungal contaminants in highly filtered hospital air is described. The sampling method addresses the limitations often associated with culturable methods, including the collection of large sample volumes, the use of sampling times of several hours or more, and accommodates the cost-effective application of multiple methods of analysis.

METHODS

Microscopic Analysis

The BA cassettes contained a mixed cellulose ester (MCE) filter (Millipore Corp, Bedford, MA) with a pore size of 3.0 um, which was supported by a cellulose pad. Samples were collected at airflow rates between 1 - 3 lpm using either a Model 224-PCXR8 (SKC, Inc., Eighty Four, PA), Basic 12 (A. P. Buck,), or a high-volume Gast pump (EMS, Charleston, SC) Samples were typically collected for 2 - 4 hours.

Following sample collection, the cassette was opened and the filter and pad were placed on a clean surface. The two sample traces were separated by cutting the filter in half

using an Xacto Knife with a ½ inch blade. One sample trace was placed on a glass slide, and the slide inserted into a Quick Fix® acetone vaporizer (EMS, Charleston, SC) to collapse and clear the MCE filter. The rough surface of the MCE held the spores in place during handling and staining. The sample was then stained with lactophenol cotton blue, covered with a cover slip, and the entire sample trace was analyzed by light microscopy at 600x magnification. Analyses were performed using a Nikon Labophot-2 or Alphaphot PCM microscope (Nikon America, NY, NY) equipped with a 10x eyepiece and planachromat 10x, 20x and 60x objectives

RESULTS

Many of the airborne samples collected in highly filtered air were censored, or below the LOD. Since excluding these data would have both biased the results and severely limited the comparisons, the data were treated as having been drawn from a lognormal distribution of concentrations; and censored data were entered into the database as the LOD/2. Therefore, the reported geometric mean (GM) concentrations are artificially high for highly censored data.

The 177 samples described in Table 1 were analyzed by microscopy. The seismic chamber beneath one of the seven hospitals was sampled because of the possibility that spores could infiltrate wall cavities. From 60 % to 100 % of the samples collected in the various hospital areas and analyzed by microscopy had *Asp/Pen* spore concentrations that were censored, or below the LOD. The sample areas in the table are in descending order of GM concentrations. The GM concentrations were in a relatively narrow range from a high of 2.8 spores/m^3 to a low of 0.8 spores/m^3

Table 1. Samples collected in various areas of seven hospitals and analyzed by microscopy; concentrations of *Asp/Pen* spores in sp/m^3.

AREA	SAMPLES	BELOW LOD	MIN	MAX	GM	GSD	AVG
Seismic Chamber	**15**	**1**	**42**	**1,619**	**273**	**2.5**	**398**
Surgical Support	**10**	**6**	**0**	**78**	**2.8**	**9.6**	**17.4**
Interior Spaces	**43**	**30**	**0**	**222**	**2.1**	**9.4**	**24.7**
Out Patient	**19**	**13**	**0**	**108**	**1.6**	**2.7**	**13.6**
ICU	**62**	**47**	**0**	**218**	**1.1**	**4.5**	**6.9**
Operating Rooms	**20**	**17**	**0**	**15**	**0.8**	**2.7**	**1.7**
Sterile Processing	**8**	**8**	**0**	**0**	**NA**	**NA**	**0**

The data in Table 2 describe the sample results for 42 samples that were collected in seven hospitals and analyzed by QPCR using a panel of 24 primers; although only the results for *Aspergillus* and *Penicillium* are reported in the table. Surgical Support included post-operative recovery, MRI suite, catheter laboratory, Gamma Knife, radiation/Oncology, and day surgery. Out Patient included patient waiting areas and emergency rooms; and Interior Spaces included lobbies, hallways, elevators and offices. The sample areas in the table are in descending order of geometric mean (GM)

concentrations. The GM concentration was highest in Surgical Support, intermediate in Interior Spaces, and lowest in ICU's, Operating Rooms, Out Patient, and Oncology.

Table 2. Samples collected in various areas of seven hospitals and analyzed by QPCR; concentrations of *Aspergillus* and *Penicillium* spores in sp eq/m^3.

AREA	SAMPLES	BELOW LOD	MIN	MAX	GM	GSD	AVG
Surgical Support	**4**	**0**	**36**	**1,794**	**225**	**3.1**	**635**
Interior Spaces	**4**	**2**	**3**	**1,070**	**30.3**	**17.5**	**291**
ICU	**10**	**3**	**0**	**74**	**6.5**	**6.4**	**23.5**
Operating Rooms	**11**	**4**	**0**	**31**	**1.7**	**3.7**	**4.5**
Out Patient	**6**	**2**	**0**	**8**	**1.4**	**2.9**	**2.3**
Oncology	**5**	**3**	**0**	**9**	**1**	**3.5**	**2.3**
Sterile Processing	**2**	**2**	**0**	**0**	**NA**	**NA**	**0**

The 12 fungi listed in Table 3 were detected in the 42 QPCR samples collected in the various hospital environments. These fungi were collected in baseline samples obtained in the seven hospitals, and may be representative of fungi commonly present in healthcare facilities in southern California. *A. niger*, which may be associated with nosocomial infections, was detected in 15 % of the samples, *A. flavus* was detected in 1.9 % of the samples, and *A. fumigatus* was not detected. A primer for *A. terreus* was not available, and was not included in the QPCR panel.

Table 3. Frequency of occurrence of fungi detected in hospital QPCR samples.

FUNGI	SAMPLES	PERCENT
None Detected	**15**	**27.8**
Aspergillus penicillioides	**15**	**27.8**
Aspergillus niger	**8**	**14.8**
Penicillium chrysogenum	**3**	**5.6**
Eurotium amstelodami	**3**	**5.6**
Aspergillus sydowii	**2**	**3.7**
Scopulariopsis chartarum	**2**	**3.7**
Aspergillus ustus	**1**	**1.9**
Aspergillus flavus	**1**	**1.9**
Aspergillus vresicolor	**1**	**1.9**
Penccillium corylophilum	**1**	**1.9**
Penicillium variabile	**1**	**1.9**
Scopularopsis brevicaulis	**1**	**1.9**

Table 4 contains the *Asp* and *Pen* concentrations detected in five of 13 newly constructed Operating Rooms that were sampled to commission them for occupancy. Essentially neither spores nor spore equivalents were detected in the remaining nine OR's not included in the table. The differences between the results obtained for microscopy and QPCR for these five OR's were sufficiently large to affect the interpretation of the sample results. Microscopy indicated that the five OR's were in an acceptable condition.

However, the QPCR results indicated the presence of substantial amounts of spore equivalents.

Table 4. Commissioning samples for five of 13 newly constructed Operating Rooms; concentrations of *Asp* & *Pen* spores (microscopy) and spore equivalents (QPCR) per sample and per cubic meter;.

OR	SPORES	SP / M^3	SP EQ	SP EQ / M^3
1	0	0	31	220
2	0	0	7	74
3	1	6	7	65
4	0	0	4	60
5	0	0	3	46

The corner of a typical BA sample trace at 100X magnification is illustrated in Photo 1. The edges of the sample trace were bounded by compression ridges below the sample slit, and the boundary of the sample trace was sharply defined. As a result, the outline of the sample trace was sharply defined. The spores were retained within the area of the rectangular sample trace, and remained within the sample trace after transportation, handling and staining. This feature of the BA minimizes the area of filter that is required for analysis.

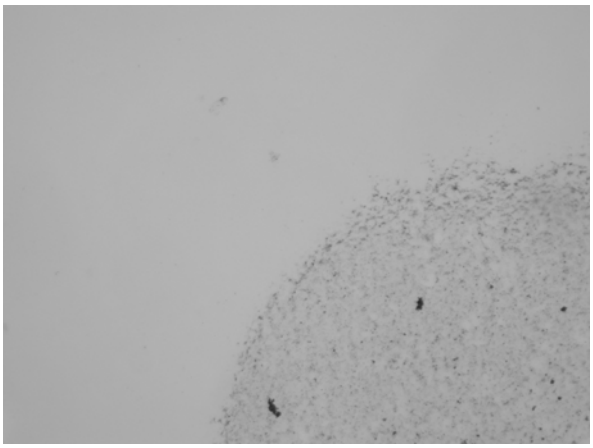

Photo 1. Example of a typical sharply defined BA sample trace at 100X magnification.

Photo 2 illustrates the portion of the 25 mm MCE filter that is typically analyzed. The area of a 25 mm filter is about 490 mm^2, while the area of each sample trace is 9.35 mm^2, which represents 1.9 % of the total filter area. Only one of the duplicate sample traces collected with the BA filter cassette, contained on one-half of the filter, was submitted for QPCR analysis. Only the relatively small area of filter surrounding the sample trace, typically less than 4 % of the total filter area, was required for analysis. The ability to discard 96 % of the filter allowed smaller wash volumes to be used, increasing the sensitivity of the method; and minimized interferences due to the presence of contaminant fungi on the filter.

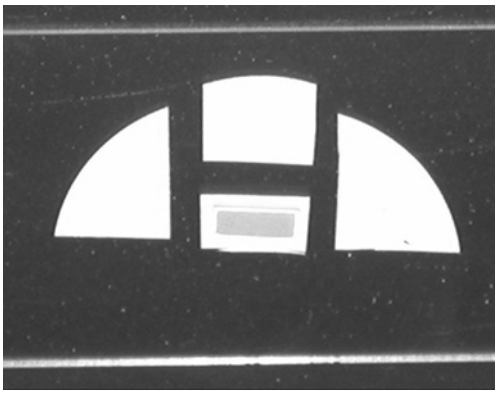

Photo 2. Relative size of a 9.35 mm^2 Bi-Air sample trace compared to the490 mm^2 total area of a 25 mm filter.

The data in Table 5 compare example concentrations of spore equivalents by QPCR and spores by microscopy that would be reported for a hypothetical 168 liter sample. The sensitivity of QPCR analysis varies between fungi, as illustrated in Table 6. Therefore, direct comparisons between microscopy and QPCR were based on spores/spore-equivalents per sample rather than spores/spore-equivalents per cubic meter of air. These data were expected to be reasonably comparable since the analyses were performed on duplicate samples.

Table 5. Comparison of fungal concentrations reported by QPCR and microscopy for an example 168 liter sample.

FUNGI	SP EQ / SAMPLE	SP EQ / M^3	SP / SAMPLE	SP / M^3
Scopulariopsis	**1**	**3**	**1**	**6**
A. flavus	**1**	**6**	**1**	**6**
P. chrysogenum	**1**	**27**	**1**	**6**
A. versicolor	**1**	**38**	**1**	**6**

Filters, although typically free of fungal spores detectable by microscopy, may contain fragments of spores and hyphae detectable by QPCR analysis. Table 6 contains the contaminant fungi and their concentrations detected by QPCR on six MCE filters that were submitted as blanks. The data in Table 6 were obtained by analyzing the entire 25 mm filter, with an approximate surface area of 490 mm^2. The data in the last column of Table 6 are the concentrations of contaminant fungi contained in the 4 % of the filter area necessary for analysis by QPCR when analyzing a BA sample trace, as illustrated in Photo 1.

Table 6. Contaminant fungi detected on six 25 mm MCE filters submitted as QPCR blanks.

SAMPLE NUMBER	TOTAL SP EQ	SP EQ / 100 mm^2	FUNGI DETECTED	SP EQ / FILTER	SP EQ / TRACE
1	0	0	None Detected	0	0
2	0	0	None Detected	0	0
3	3	0.6	*Asp. penicillioides*	3	0.1
4	27	5.5	*Pen. brevicompactum*	20	0.8
			Asp. fumigatus	5	0.2
			Scop. Chartartum	2	0.1
5	37	7.6	*Clad. Herbarum*	18	0.7
			Mucor. amphibiorum	12	0.5
			Clad. cladosporioides	7	0.3
6	156	31.8	*Pen. variabile*	63	2.4
			Aureo. pullulans	63	2.4
			Alt. alternate	7	0.3
			Asp. penicillioides	4	0.2

Figure 1 is a direct comparison of airborne *Aspergillus* and *Penicillium* concentrations in the 36 hospital samples analyzed by both QPCR and microscopy. QPCR not only detected fungal spores, but also fragments of spores and hyphae. Therefore, the concentrations of spore equivalents in Figure 1 that were derived from QPCR analysis were often higher than the corresponding spore counts based on microscopic analysis.

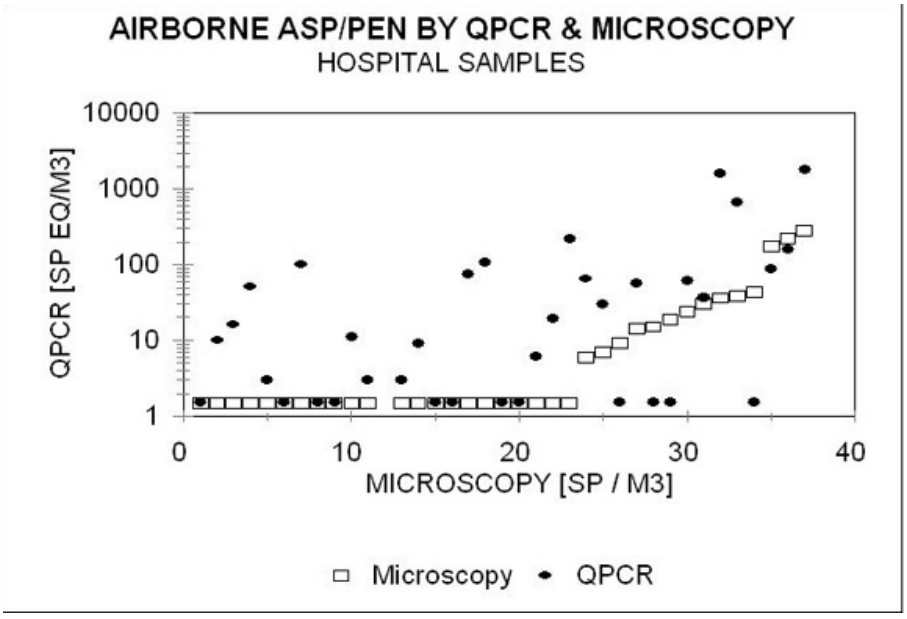

Figure 1. A direct comparison of airborne *Aspergillus* and *Penicillium* concentrations in the 36 hospital samples analyzed by both QPCR and microscopy.

The data in Figure 2 contain five residential samples in addition to 31 hospital samples. The five residential samples were included to extend the concentration range, since the majority of the hospital samples had relatively low concentrations of *Aspergillus* and *Penicillium* spores. In addition, the data were presented using log scales because most of the data were grouped near zero. The coefficient of correlation (r-value) was 0.93, suggesting a good correlation between the two methods of analysis. The concentration of spore equivalents was higher than the spore count in eight of the ten noncensored samples, which was the expected relationship. However, *Asp/Pen* spores were reported in four samples in which spore equivalents were not detected.

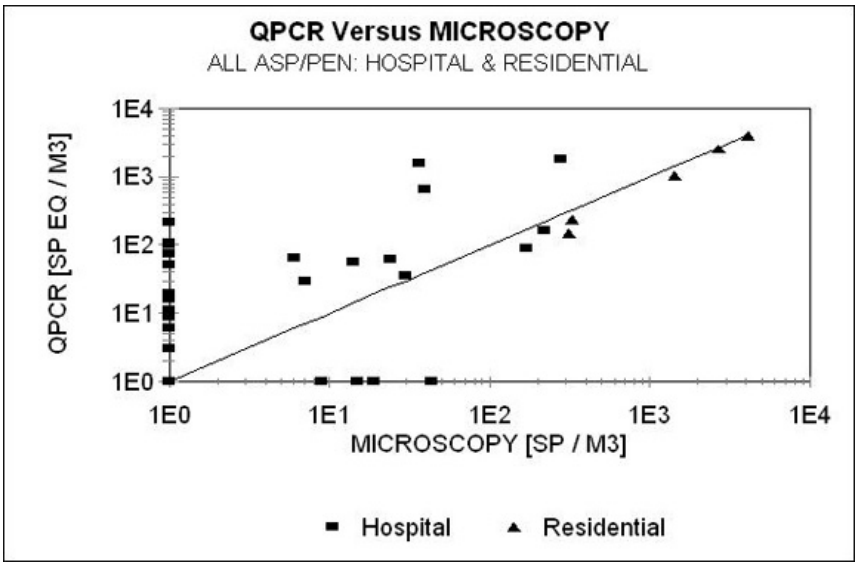

Figure 2. The correlation between QPCR and microscopic methods for *Aspergillus* and *Penicillium* concentrations collected in both hospital and residential environments.

DISCUSSION

Characterization of Hospital Environments

The data in Table 1 are microscopic spore counts, in spores/m^3, for these same spaces. Based on spore counts, the GM concentration was essentially 2 or less in Sterile Processing, Operating Rooms, ICU's, Out Patient, and Interior Spaces.; and less than 3 spores/m^3 in Surgical Support. Based on spore counts, all the areas that were sampled in the seven hospitals were essentially in control. Anecdotally, the *Asp/Pen* spore counts in ICU's and in post-op tended to increase with the number of visitors present.

The data in Table 2 are a comparison of sp-eq/m^3 as measured by QPCR. The data compare the *Asp* and *Pen* concentrations detected in various representative spaces within seven hospitals. GM concentrations in Sterile Processing, Oncology, Out Patient areas, and Operating Rooms were less than 2 sp-eq/m^3; the GM concentration in ICU's was 6.5 sp-eq/m^3; and general Interior Spaces had a GM of 30.3 sp-eq/m^3. However, Surgical

Support, which included post-operative recovery, had a relatively high GM of 225 sp-eq/m^3. Therefore, the potential existed for transferring a patient from a relatively sterile Operating Room into a less sterile environment in Post-Operative Recovery.

Table 3 contains the fungi that were detected in the seven southern California hospitals by QPCR analysis. The second most frequently detected fungus was *A. niger, A. flavus* was detected infrequently, and *A. fumigatus* was not detected in any of the samples. A PCR primer for *A. terreus* was not available, so this fungus could not be detected by QPCR. The fungi detected in a particular hospital would be expected to vary temporally, spatially, and with changes in conditions. However, except possibly for *A. niger*, these baseline samples suggested the hospital environments posed limited risk to patients.

Comparison of Microscopy and QPCR

One series of samples was collected as part of the commissioning of 13 newly constructed Operating Rooms. The data for five of the OR's are contained in Table 4. Both the spore counts by microscopy and spore-equivalents by QPCR indicated that nine of the OR's were essentially free of *Asp* and *Pen* contaminants. The environments in the five OR's described in Table 4 were acceptable based on the microscopic analysis of 100 % of the initial BA sample trace, but were problematic based on the QPCR analysis of the second sample trace. However, QPCR not only detects spores, but hyphael fragments and spore fragments. Therefore, it was expected to be a more sensitive method of analysis.

The differences routinely observed between the two methods of analysis made it necessary to adopt one methodology as the primary basis for assessing exposures. Prior to the collection of the data, microscopic analysis had been identified as the primary basis for performing exposure assessments, while QPCR had been identified as the method most appropriate for performing risk assessments. Therefore, the detection of only one Asp/Pen spore in the five samples resulted in a negative exposure assessment for the five OR's.

Figure 1 is a rank order of the spore and spore-equivalent concentrations for 36 baseline samples. The concentration of spore equivalents was higher than the concentration of spores in 18 of the samples in which the spore-equivalent concentrations exceeded 10 sp-eq/m^3. In addition, 22 of the samples in Figure 1 were censored by microscopy, while only eight of those same samples were censored by QPCR. Since QPCR was expected to be a more sensitive method of analysis, this was the expected relationship.

However, four samples in which spore concentrations of up to 43 spores/m^3 were detected by microscopy were censored by QPCR; which was not the expected relationship. This was apparently due to the uneven distribution of spores and/or spore fragments between the two BA sample traces. At the very low spore concentrations typical of highly filtered air, one sample trace may have captured the entire fungal loading.

Microscopy and QPCR also differ in the way results are reported. For example, the comparable results for a hypothetical 168 liter sample are illustrated in Table 5. The spore concentration as measured by microscopy is independent of spore type. In this example, the detection of one spore would be reported as an airborne concentration of 6 spores/m^3, independent of the type of spore detected. However, the sensitivity of QPCR varies with spore type. As indicated in this example, one sp-eq of *Scopulariopsis* would be reported as 3 sp-eq/m^3, while one sp-eq of *A. versicolor* would be reported as 38 sp-eq/m^3. This may be an additional reason to expect concentrations reported as sp-eq/m^3 by QPCR to vary from those reported as spores/m^3 by microscopy.

Figure 2 illustrates the correlation between sp-eq/m^3 and spores/m^3 for 31 samples collected in hospitals, and for an additional five samples collected in residential properties. Many of the hospital samples were clustered at low concentrations, so the axes were presented using log scales. In addition, the residential samples were included in the correlation to extend the concentration range. The coefficient of correlation (r-value) for the 36 samples was 0.93, indicating good correlation between the two methods of analysis. The majority of the noncensored QPCR samples were either near the line of best fit or above it, which was consistent with the expectations based on thee data in Table 5.

Sampling Method

MCE filters are sterile, and essentially free of fungal spores when examined by microscopy. In addition, 16 of 42 field samples (38 %), which were analyzed by QPCR using the entire half of a 25 mm filter, did not contain detectable concentrations of sp-eq. These results suggested that filter contamination was not a substantial issue. However, MCE filters are not always free of sp-eq as measured by QPCR. Table 6 indicates the results for six blank 25 mm MCE filters analyzed by QPCR. Total spore equivalents ranged from 0 to 156 sp-eq per filter, with *A. fumigatus* detected on one filter. Although the sample size was relatively small, the results suggested that the presence of contaminant sp-eq should be expected when submitting MCE filters for QPCR analysis; that the concentrations of contaminant sp-eq varied substantially between filters, limiting the utility of submitting blanks; and emphasized the importance of minimizing the filter area subjected to analysis.

Minimizing the area of filter subjected to analysis is an important characteristic of the sampling device used to collect a sample for QPCR analysis; and the BA filter cassette is one sampling device with this characteristic. Photo 1 illustrates one corner of a BA sample trace viewed at 100X magnification. The sample area is sharply defined, with the sample deposited within the boundaries of the sample trace. The lack of dispersion results in the sample being deposited onto a defined area of 9.35 mm^2, which is less than 2 % of the total filter area. Therefore, less than 4 % of the total filter area is required for QPCR analysis, as illustrated in Photo 2.

The average concentration of contaminant spores for the six blank 25 mm MCE filters was 37 sp-eq per filter. These data suggested the expected average concentration of sp-

eq per sample would be: 87 for a 37 mm filter; 19 for 50 % of a 25 mm filter; and 1.4 for a BA sample trace assuming 4 % of the filter was analyzed. Therefore, the small area of a BA sample trace would be expected to provide a substantial advantage when attempting to detect low concentrations of contaminant spores in highly filtered air when using QPCR analysis.

The second characteristic of the BA that allowed the described sampling protocol to be implemented are the duplicate sample traces. The dual samples allow the initial sample trace to be analyzed by microscopy, resulting in a rapid, cost-effective exposure assessment based on the presence or absence of *Asp/Pen* like spores. The second sample trace may then be submitted for culturing on multiple media; or for a more rapid analysis by QPCR whenever a risk assessment is required

Example Field Investigations

The sampling protocol described in this article has been used to collect periodic baseline samples, as part of incident investigations, and to collect post-remediation verification samples following mold remediations. Baseline BA samples were collected on a quarterly basis at participating hospitals. These samples were typically collected at airflow rates of 1-2 lpm for sampling periods of 1-3 hours. The results for baseline samples are described in Table 1 and Table 2. The advantage of this protocol for baseline samples is the smaller variances associated with the collection of time-weighted average (TWA) samples compared to short-term "grab" samples. The reduced variability of the data provided greater confidence when assessing the status of the sampled spaces.

An example of an incident investigation is illustrated by a suspect Operating Room. As the result of the recommended inspection by Facilities following the investigation, two walls of the OR were found to be contaminated and were remediated. Because physicians were reluctant to use the facility, a request was made by Infection Control to assess the OR for fungal contaminants. A 3-hour BA sample was collected at an airflow rate of 3 lpm, resulting in a LOD of 1.9 spores/m^3. Four *Asp/Pen* like spores and one *Stachybotrys* spore were detected in the sample, confirming that a fungal amplification site was affecting the OR. First, this example illustrates the concept that the investigator is attempting to detect a "rare event" when sampling for contaminant spores in highly filtered air. This result was equivalent to detecting one contaminant spore an average of every 36 minutes; or one spore per 108 liters of sampled air. Second, the LOD required to detect the problem in this OR was quite low. *Stachybotrys* was detected at a concentration of 1.9 spores/m^3, and *Asp/Pen* spores were detected at a concentration of 7.6 spores/m^3. Unless an extended-period TWA sample had been collected, or multiple grab samples had been collected over an extended period of time, the probability of obtaining a false negative, or failing to detect an existing problem, would have been significant.

An example of applying the described sampling protocol to post-remediation verification sampling is illustrated by a mold remediation in an organ transplant ICU ward. The referenced HICPAC document suggests that a concentration of 0.9 cfu/m^3 of *A. fumigatus*

may still be problematic. Based on this document, the relevant parties agreed prior to the remediation that a concentration of 0.8 spores/m^3 or less of *Asp/Pen* like spores would be the acceptance criterion for airborne samples. This LOD required the collection of 1,260 liters of air per sample, which was collected at an airflow rate of 3 lpm over a period of seven hours using BA cassettes.

A total of 13 sampling locations were required to assess the ICU ward. One room had an *Asp/Pen* concentration of 0.8 spores/m^3 and was failed. It was re-cleaned and then passed; requiring a total of 14 BA samples to be collected. In comparison, either nine 5-minute N6 culturable samples or nine 10-minute slit impaction samples would have had to have been collected at each of the 14 sampling locations in order to achieve an LOD of 0.8 spores/m^3; and therefore the same level of protection. Collecting 14 BA samples versus 126 samples using an alternative sampling device provided a significant cost advantage.

SUMMARY

A sampling protocol has been described for the detection of airborne fungal spores and/or spore-equivalents in highly filtered hospital air. The protocol is simple to implement, and is applicable to the collection of routine baseline, incident investigation, and post-remediation samples. The use of the dual samples collected using the BA filter cassette as the sampling device allows a rapid exposure assessment to be performed using microscopy. When a risk assessment is required, those samples with an elevated concentration of *Asp/Pen* like spores may be submitted for the rapid identification of fungal spores to species using QPCR.

The BA filter cassette may be used to collect extended-period samples of seven hours or more, with sample volumes exceeding 1,000 liters, resulting in a LOD of 0.8 spores/m^3 or less. The longer sampling times minimize variability, providing more confidence in data interpretation. Second, the ability to detect very low concentrations of spores minimizes the probability of obtaining a false negative, or not detecting a problem when one actually exists. Finally, the longer sampling times and larger sample volumes possible with the BA cassette reduces the number of samples required to achieve a given LOD, substantially reducing sampling costs.

Finally, the small area of filter analyzed when using the BA filter cassette minimizes the effect of contaminant sp-eq when performing QPCR analysis. The BA concentrates the sample onto less than 4 % of the total filter area, allowing 96 % of the filter to be discarded prior to analysis by QPCR.