

Environmental Sampling for Meaningful Interpretation of Data in Hospitals

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Introduction

In an effort to problem solve infection control (IC) issues or commission new or renovated space, air sampling is often considered an important factor. How such data is interpreted into useful information still remains confusing and of uncertain value. Airborne spores using either culture or microscopic techniques have been the most common data for analysis. The IC staff and the contractor consider the value of culture methods, which require shipping and up to 14 days for analysis and interpretation. Also, microscopic methods for collecting ambient particles while providing timely results does not distinguish pathogens from common airborne spores (i.e., *Aspergillus fumigatus* v. *Penicillium* species).

Regardless of the methods for detection of airborne fungal spores or bacteria, the range of normal values begs for appropriate interpretation of results. Exposure to spores and human-derived bacteria are a common reality that is accepted in IC practice. Exposure of immunocompromised patients to opportunistic fungi (see Chapter Four) is another factor that requires protective measures. Because we know that such exposures are problematic for opportunistic infections, protective environments provide a haven while the patient's immune system is reconstituted. Under such circumstances, assurance of pressure at >0.01 in wg, filtration efficacy and air exchanges are necessary in order to protect patients from health care-associated disease caused by airborne fungi.¹

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This chapter will not dwell on these ventilation parameters, but understand that when sampling air for opportunistic fungi, ventilation deficiencies may affect the air quality content. Evident deficiencies in the ventilation may be causing transient exposures to opportunistic airborne pathogenic fungi. In other words, if depressurization of protective areas exists, airborne fungi may migrate to the compromised host via air movement. Hospitals treating severely immunocompromised patients need a surveillance plan to ensure ventilation management so that air quality content is controlled during normal and reconstruction conditions.

When to sample

Take an environmental sample for several reasons: When the facility with special ventilation requirements (protective environment) is initially opened. This would include a bone-marrow transplant unit or perhaps operating rooms. When there is water damage to determine whether drying up or cleaning up of the water damage is complete. Be sure there is no residual microbial contamination.

Commissioning sampling should occur just prior to occupancy; however, the assurance that the ventilation is functioning is critical to the acceptance criteria for safe indoor air quality (IAQ). The best time to sample is after all construction-related cleaning is complete and before occupancy. However, it cannot coincide with the fire management testing. The fire management test requires depressurization testing for smoke removal. During these tests, the building or area is depressurized allowing for particles to migrate into the affected test area. Such environmental conditions would skew particle sampling to a worst-case scenario. Be aware of other tests that could interfere with sampling.

Outbreak investigation analysis may also provide useful epidemiological data. However, biological considerations must be understood in order to grasp the exposure potential. For example, spore exposure is the result of either improperly placed filtration or a local source of fungal spore accumulation. The local source release can result in a short-term, high-dose exposure, which dissipates quickly. To look for such airborne contamination weeks after the event may not be productive. Such investigations should include an evaluation of maintenance or other disruptive (construction) activity timeline that may have occurred in the areas being investigated.

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In addition, surface sampling for the residual spores on return/exhaust grills or fleecy indoor surfaces may hold clues from residual spore levels.

Routine surveillance sampling reassures us that mechanical and operational procedures are working. I have demonstrated the existence of short-term, high-dose release of fungi from a variety of sources.² Such events most often are not captured during sampling. It is not necessary to sample for such events, therefore preventing the escape of such short-term aerosols is essential for exposure control. These steps provide the greatest benefit for IC. Following protocols for containment with appropriate depressurized conditions using HEPA filtration and other procedures will do more for preventing infections than sampling the air for airborne fungi.

Sampling during construction is often thought to provide meaningful data for preventing infections. In fact, the sample results provide data too late to do any good if culture methods are used. Sampling of air before a project should provide information needed to avoid ventilation deficiencies. If these deficiencies are found, they can be corrected. (*Author's note: a building ventilation deficiency that jeopardizes patient safety should not be detected during a construction project.*) For example, it would be best to know about particles in air (viable and nonviable) during the preconstruction phase to ensure proper filter installation for the filters if external construction is planned. During samples it is important to verify the level of indoor airborne fungal levels compared to outside levels.

Air sampling data has questionable meaning if it is not compared to other indoor areas and the outside. Filters, windows, and air exchanges help to reduce the levels of fungal content to achieve an indoor/outdoor ration of less than one. The existing ventilation conditions help to interpret the data. The risk assessment for respective areas depends on their patients' susceptibility and the ultimate impact of the work being planned.

Verification of existing conditions is an important part of the preconstruction process because if a ventilation deficiency exists (filters not properly installed), that area may be prone to opportunistic spore infiltration. The goal is to make sure the building systems are operating close to specification of that mechanical system. Providing corrective measures on deficiencies will lessen the liability of those deficiencies if infections occur and litigation results.

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Internal projects require considerations for building penetrations. Major breeches in buildings include doors, air intakes, and windows. Whether you evaluate the potential deficiencies with a particle counter or other air sampler requires knowledge of the building performance to develop the baseline. Baseline data for an external project depends of the ability of the filtration systems to remove the incoming particles as a percent efficiency. If the filters are said to be 90% efficient, will they remove 90% of incoming particles? This of course depends on the particle size. While spores are two to five microns, the presence of human activity may bias this particle size if a nonviable particle counter is used. The >0.5 micron particle size may be a better means of determining the efficacy of filtration systems. The particle counter can be an excellent means of determining whether the general conditions in the facility space in question are operating properly. The viable air sampler can be used to determine whether local contamination is present. The disadvantage of viable sampling data is due to culture results coming as long as five to 14 days later. What do you do with the information after that much delay?

Other sampling methods include using cassette impactors, which can collect the particles on a membrane surface for microscopic counting. Although this method still has a time delay, albeit much shortened (less than 24 hours), it has certain deficiencies for health care safety clearance. For example, the differentiating between *Penicillium* species and *Aspergillus* species spores is difficult. However, the bottom line is that this method provides preliminary clearance for construction projects. The prudent clearance requirements for occupancy would require culture clearance because the microscopic cassette methods do not provide viable information, which may be an important consideration for an area about to house immunocompromised patients.

Previous outbreak analysis experience has revealed large amounts of contamination on surfaces and not in the air. Carefully conduct the sampling for occupancy clearance before patients are allowed in after new construction or water damage remediation in special immunocompromised patient units.

Sampling process

Before sampling air for microbial content, assurance of high-quality samples require that the local or building ventilation parameters are working as specified. The concept for problem solving infections includes the analysis for an area to be pressurized or depressurized according to operational conditions. If the ventilation conditions are not known at the time of sampling, additional evaluation may be necessary to identify the potential source of isolates recovered from the sampling. Spores from fungi are propagated during biodegradation conditions outdoors or indoors.

Indoor sources are created from conditions favorable to growth due to water availability, food, and temperature. The spores, once developed, are passively liberated when disturbed by disruption (construction or maintenance), draft, or vibration. Differentiation of these sources requires a ventilation assessment, which can be conducted with the use of a pressure gauge and particle counter. Migration of particles on air currents is possible with pressure differences as described by Thio (et.al) in an outbreak at John's Hopkins Hospital.³ Data collected during this evaluation of an *Aspergillosis* outbreak showed a high particle content inside the hospital. Also data showed fungal pathogens on surfaces and in relatively low concentrations from the ambient hospital air. However, the particle content of supply air in that building was very low compared with outside levels.

The high ambient content occurred because the building was depressurized allowing particles to migrate indoors. Most likely, the infectious particles migrated indoors during extensive excavation of a nearby construction site. Comprehensive environmental sampling requires an understanding of ventilation conditions and local sources when investigating such clusters of infection by airborne infectious agents.

Sampling devices

A variety of sampling devices and methods may be needed to evaluate indoor air. Which methods provide the most meaningful information requires an understanding of the health

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care environment. For example, the most common sieve impactor samples air at 28.3 l/min which may undersample the local air in a highly filtered patient care environment.

Clusters of infections have been associated with very low levels of airborne fungi. Rhame et. al.⁴ at the University of Minnesota attributed a 15% infection rate with 0.9 CFU/m³ *Aspergillus* levels. Arnow et al⁵ also demonstrated a 5% infection rate with 1 to 2 CFU/m³ of *Aspergillus flavus* in air at the University of Chicago Hospital. For example, only five-minute samples were taken at 28.3 l/min (140 liters), air sampling probably would not have collected the implicated pathogen. Therefore, high volumes of air need to be collected for microbe recovery.

The American Society of Microbiology Air Cultures for Fungi⁶ recommends a high-volume sample totaling 1,000 liters, which increases the chances for collecting the pathogen. Some samplers (see Figure 8.1 at the end of this chapter) do collect larger volumes of air without drying the surface of the media, but those samples must be collected with the appropriate device (slit v. sieve impactors).

Collection of viable samples will ensure culture-proven isolates. The total allergen content can be collected with the cassette method, which should also discriminate the rank order of particles (clean to cleanest). However, the viable and type organism certainty are not determined. Media considerations must also be factored into the collection. Malt extract agar with inhibitor for bacteria is a good choice to provide a broad spectrum of growth potential for a variety of fungi. Evaluate Xerophilic microbes using DG-18 with inhibitor. This media prevents overgrowth of the isolates allowing for easier analysis in contaminated environments. Due to the relatively clean, highly filtered hospital environment, the malt extract agar (MEA) is a good overall media for health care air analysis.⁷

Likewise, it becomes difficult to determine the best incubation temperature. Ideal incubation for mesophilic or thermotolerant organisms is the question. The room temperature fungi are more common than body temperature fungi. The pathogen recovery is also greater at body temperature. The problem is more difficult with analysis at the lower temperature due to potential pathogen masking from other airborne fungi recovered. Ideally dual sampling would allow for both room temperature (25°C) and body temperature (37°C) incubation. The room

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temperature provides the gross analysis to determine the efficacy of ventilation systems while the body temperature analysis would allow for pathogen discovery. The higher temperature incubation does not provide the relatively high isolate recovery due to natural temperature selectivity. Isolate recovery is selecting for those that grow at body temperatures. Room temperature versus body temperature may vary up to one order of magnitude with room temperature organisms being more abundant.

As previously stated, the nonviable collection on cassette or particle counter can provide supplemental data for analysis. An optical particle counter can analyze the nonviable particles by size. Size analysis can be confusing, especially if the optical particle counter has size differentiation based on 0.3 μm and larger particles. The 0.3 μm particle in theory is the most difficult to filter. The 0.5 μm and larger particle provide a basis for clean room standards and is a logical choice for air quality measurement. The disadvantage with most particle counters relates to collection time for comparison, which usually amounts to collecting 0.1 cu ft to 1 cu ft of air.

The sampling time for this can result in large fluctuations in air particle content. The use of a condensation particle counter can provide instantaneous updates of air particle content. This allows for real-time analysis of air quality after filters or around windows to check for the infiltration of particles. The particles collected with the ultrafine real time condensation counter are very small—0.02 μm to 1.0 μm —however, these particles are removed via filtration by diffusion, therefore the filter efficacy is maintained and reflective of particle removal.

Real-time analysis allows for quick evaluation of source potential and response, which can ensure IC. The disadvantage of particle counting is not being able to identify the particles collected. For example, the measurement of the filter efficiency can demonstrate that leakage filters are not properly installed as part of a preconstruction risk assessment. During outbreak and infection control risk analysis, such breeches in the filters have been found using visual and particle count analysis. Validation of any data may require repeat sampling in order to ensure a consistent deviation from baseline data.

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Adjunct to these analyses is the use of pressure-sensing devices to determine intensity of airflow movement. Sensitive monitoring devices detect the airflow velocity between areas needing such control. The airflow velocity at 0.25 Pascals (0.001 in water gauge) is about 120 lfpm while the velocity at 2.5 Pascals (0.01 in wg) is about 400 lfpm. This difference represents air velocity capable of moving spores in the airflow into critical care areas. Monitoring of these air differentials during construction becomes a major factor for preventing contamination by providing clean-to-dirty airflow. These data from environmental testing are useful for problem solving or verifying ventilation parameters.⁸

Interpretation of data

Interpretation of data becomes a major factor when evaluating health care environments. Important to understand is the fact that many of these fungal pathogens are very common in outdoor air. We cannot expect to abandon a patient care environment because of 1 CFU/m³ in a sample. Response should be according to several sample data results. The first consideration is the rank order of sample isolates collected. Sampling must include comparison data. This data should demonstrate clean to cleanest environments. Compare all samples to outdoor data. The highest quality air should be in the area with the best filtration and appropriate ventilation requirements. This is needed to provide an indoor/outdoor ratio of <1. The normal data would provide an indoor/outdoor (I/O) ratio of < 1.

In nonagriculture conditions, this I/O ratio may not be as obvious as in agricultural conditions. Nonagriculture conditions would include winter with snow cover, oceanfront, or desert locations. The I/O is more obvious for room temperature incubation cultures. The I/O can also be seen with cassette impaction samples or particle counts and can be a factor in evaluating a critical indoor environment.

The qualitative analysis of the microbes is important from two factors. Outdoor samples are generally heterogeneous in nature, meaning the presence of many types of microbes. Indoor samples often reflect similar heterogeneous population but in lesser numbers with the I/O <1.

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If pathogens are recovered, a single colony may represent a random event. Two or more cfu per sample may represent a breach in ventilation or a local source. Resampling should occur after the recovery of two more pathogen isolates. The average sampling results should indicate pathogen levels at $< 1 \text{ CFU/m}^3$ or $< 1 \text{ cfu/plate}$ for surface samples. (See Figure 8.2 for an interpretation of microbiology data.)

FIGURE 8.1

AIR SAMPLERS FOR FUNGAL SPORES

Samples types	Principles	Rate	Comments
Sieve impactor	Impactor on agar plate	28-180	Low volume precludes short-term collection that might relate to specific activities; effective for high concentrations of spores
Slit impactor	Impaction on rotating agar plate	30-700	High volume allows short-term collections that might relate spore acrosols to specific activities; effective for low concentrations of spores; quantification may be compromised at high levels of spores.
Centrifugal Impactor	Impaction on plastic strips containing agar media	40	Calibration difficult, thus limited to relative determinations
Impingers (glass)	Impingement into liquids	12.5	Low-volume sampling rates and tendency to disrupt clumps limits application to non-clinical sampling.
Filters (cassette)	Filtration of air through 0.2 um pore size filters	1-2 or 140-1,400	Not practical for viable microbes. Can provide a close to real time microscopic analysis of air quality. Does not differential between spore similar in size & shape
Settling plates	Gravity	Setting rate of spores	Most pathogenic spores are too small and buoyant to settle. Lack of quantification severely limits sampling method

FIGURE 8.2

INTERPRETATION OF MICROBIOLOGY DATA

- **Rank order analysis**
 - Lowest counts in the areas with best filtration
 - Comparison necessary with outdoor control

- **Qualitative analysis**
 - Pathogen recovery (*Aspergillus*)
 - Homogeneous population (versus heterogeneous isolates)

- **Indoor outdoor ratio**
 - I/O <1 normal (seasonal considerations)
 - I/O >1 potential problem

- **Temperature selectivity**
 - Pathogens grow best at >35°C
 - Filtration efficacy determined at 25°C

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