

Common flaws associated with the standard spore trap analysis, and the strides the industry has made towards policing itself and validating the results of novice spore trap analysts

Airborne biological agents have always been a matter of concern in agriculture, biotechnology, industrial settings, and the indoor environment. Each of these environments presents unique exposure based on the nature of the encountered biological agent, the microbial concentrations, the modes of exposure, and the susceptibility of the exposed population. Acceptable levels of airborne microorganisms have not been established and the sampling methods and analytical techniques employed to assess airborne biocontaminants are varied and non-standardized. Selection of sampling and analytical methods depends upon the nature of the information that is sought; there is no one ideal sampling or analytical method. Combinations of sampling and analytical methods can provide a wide range of data that can be effectively adopted to different environmental settings.

Transmission of airborne microflora occurs both at a local level and over long distances. As a result numerous studies have been carried out to monitor not only disease and transmission of disease propagules in the aerobiological environment, but also allergens and mycotoxin carrying particles and recently transmission of pollen from GM crops. Actual sampling procedures may involve the passive collection of spores by gravitational deposition or sampling specific volumes of air with "active" spore-trapping devices. With most of these techniques microscopic examination of impaction surfaces is required. If accurate counts are to be obtained such techniques require considerable amounts of time and expertise. Delays in analysis of samples mean that it is difficult to use results in 'real time'. In addition, the detection of small and / or non-descript spores is required, sample identification is as yet not a realistic option. Nevertheless recent studies have demonstrated the use of existing "active spore trapping technology", notably the Burkard 7 day volumetric spore trap, marketed by Burkard Manufacturing Co. Ltd, in the development of both immunological and DNA-based for the detection and identification of target fungal air spora. Although the current spore trapping method at present is impractical for routine use, they will enable the development and validation of more rapid antibody / DNA-based spore trapping systems.

When planning for sampling, one needs to consider whether the sampler has better collection efficiency, what media to use, when and how long to sample, how many samples to take, what to compare to, which microbiology laboratory to use, and what to do with the results.

Spore trap analysis is a non-culture based sampling tool that provides an indication of the kinds and levels of total (viable and non-viable) airborne fungal (mold) spores present in the indoor environment. Direct microscopy is used to analyze the samples, providing both a qualitative and quantitative assessment of spores in the air in a short timeframe.

It is used to determine whether the mixture of airborne fungi in a building is normal and typical or indicative of moisture problems. Air sampling is also an important tool for conducting exposure assessments. Air-O-Cell sampling provides a quicker turnaround time than culture-based analysis, when rapid communication of results is essential. Often investigators use non-culturable air sampling as post-remediation clearance testing to evaluate effectiveness.

This method provides report on both raw spore count for individual spore types and total concentration expressed as particles per cubic meter (particles/m³). The proportions of each spore type are also calculated and important groupings of fungal types are summarized to facilitate interpretation of results. We also include qualitative data on pollen and skin scales for each sample.

A number of samplers for culturable fungi and for total spore counts are commercially available. These samplers are widely used by allergists, industrial hygienists, and environmental professionals. Sampling for culturable fungi includes passive techniques, such as the settling method, and volumetric samplers.

Air-O-Cell Air Sampling cassette is a sampling device designed for the rapid collection and analysis of a wide range of airborne aerosols. These include fungal spores, pollen, insect parts, skin cell fragments, fibers, and inorganic particulates. Air enters the cassette, the particles become impacted on the sampling substrate, and the air leaves through the exit orifice. The airflow and patented cassette housing is designed in such a way that the particles are distributed and deposited equally on a special glass slide contained in the cassette housing called the "trace." Benefits: Useful for initial site testing, especially if fungal growth is not visible.

Disadvantages Air-O-Cell Spore trap method

- Fungi cannot be fully speciated with this method. For example, *Aspergillus* sp. and *Penicillium* sp. are normally reported together due to the similarities in spore morphology.
- Spore viability cannot be assessed, as it is not possible to differentiate between viable and nonviable
- Sampling method is cumbersome and noisy
- Large lab-to-lab variation in identification
- Methodology not accepted by all within the industry

Sampling protocols using non-microscopic techniques have usually involve use of selective agar media or observation of disease expression following host exposure. However, these methods have limitations since they rely on passive sampling and are not universally applicable. For example, problems can arise in the detection of some slowly growing microbial species on agar, especially if the target organism occurs at low levels in the presence of large populations of other microfloral organisms. Additional problems include the incubation conditions used to express the organism after it is trapped on agar and, determining what might constitute a colony-forming unit. Using hosts to express levels of target organisms in an aerobiological population is equally problematic. For some diseases, symptoms are not expressed until weeks after infection has occurred. Therefore, it is difficult to measure accurately the degree of airborne dispersal of many airborne propagule types, when they are present in low levels.

However technological advances in diagnostics in which either antibody or nucleic acid probes are used offer the potential for developing rapid systems for detecting and quantifying target airborne material. Nevertheless the problem to date has been to develop sampling systems, and other novice methods which accommodate the use of such diagnostic probes.

Other Novice methods

Real-Time PCR is an excellent complement for current sampling strategies. If you currently use spore traps to reveal the genus names of fungi in indoor environments, you can also submit real-time PCR samples to quickly determine the species names of any

significant molds that were found on the spore traps. If you use culture techniques to determine the identity of viable organisms, you can use real-time PCR to determine the species names of those organisms sooner.

Real-Time PCR is a fast, accurate, and sensitive DNA-based analytical method for identifying and quantifying micro organisms at species level. It looks for the presence of DNA sequences that are unique to a particular mold species. It also utilizes DNA sequence detection system to monitor the presence and concentration of a specific micro organism in "REAL TIME". As a micro organism -unique sequence is detected and amplified, fluorescent signal molecules are simultaneously released and measured. No fluorescence means no target organisms are present.

Comparison of Spore trap analysis with PCR

- Air-O-Cell spore trap and PCR are both FAST tests that provide you with information regarding quantity (number of spores) and identity of spores present in your samples. Both can be completed in a day
- Air-O-Cell spore trap analysis gives you genus names (ex. *Stachybotrys*) of approximately 20 to 30 different molds and tells you if you have any *Aspergillus/ Penicillium*-like spores (*Asp/Pen* spores are very difficult to distinguish in spore traps). PCR analysis gives you genus and species names (ex. *Stachybotrys chartarum*) of all the species you choose to look for.
- Air-O-Cell spore trap analysis is subjective, depending on the expertise of the analyst. PCR analysis is not subjective since it is a DNA-based test.
- Neither Air-O-Cell nor PCR analysis can tell you if the spores in your sample are viable or not (alive or dead), which can be done only with culture analysis.
- Air-O-Cell analysis can be impossible to do if the sample is overloaded due to extended sampling time. PCR analysis can be done with as few as a single spore or as many as a billion spores.

Some of the research studies reveal that other methods like HVAC filters (Heating, Ventilating, and Air Conditioning filters), High Volume Liquid Impactors and Sensitive Enzyme Linked Immunosorbent Assay (ELISA) being used in extraction and quantification of viable fungal particles as indicators of relative airborne levels in building air.

HVAC Filters

In an evaluation performed by loading HVAC with known concentrations of fungal spores, the extraction and quantification of these previously loaded fungal spores, and the estimated number of spores loaded with the estimated number of spores recovered. A ventilation test chamber consisting of a length of 2' x 2' square ducting was used to load the filters evaluated in this research. Filters were loaded with a fungal spore aerosol generated through the use of respiratory therapy nebulizers. The nebulizers were filled with an *Aspergillus niger* spore suspension. The concentration of viable spores in the suspension was determined through serial dilution. The quantification method used to determine the fungal load on the filters involved the removal of small samples from the filter, the immersion of these samples in sterile saline, the shaking of the filter/saline combinations, and the subsequent plating of aliquots of the shaking solution onto potato dextrose agar.

In research study, by using a novel high volume liquid impaction bioaerosol sampler and sensitive enzyme-linked immunosorbent assay (ELISA) specific for that particular mycotoxin. It was possible to successfully demonstrate the presence of airborne mycotoxins produced by fungi in a controlled and six native *S. chartarum*-contaminated environments in a sampling time as short as 10 minutes. Based on standards, estimated toxin concentrations ranged from 0.45 ng/m³ to over 1.0 ng/m³ of sampled air.

Microtiter immunospore trap (MTIST)

MTIST samples at a flow rate of 57 litres of air per minute. It can be altered based on the type of microflora sampled. The MTIST uses a suction system to directly trap air spora by impaction onto the base of microtiter wells

Rapid analysis and immunoquantification of target air spora is done by using 4 x 8 well microtiter strips, specific antiserum and processed by enzyme-linked immunosorbent assay (ELISA).

MTIST device can be used to assess the presence or absence and quantity of several target air particulates either by immunoassay or by PCR-DNA based methods, which has specific probes. This is important in studies in which organisms occur as complexes in particular ecological niches. It can even be used in fields, where variable windspeeds will occur, the MTIST has been modified by addition of a wind vein and an inlet manifold which is held at 90° C to the wind.

Optimization of trapping periods has been achieved with the use of data loggers, which measure environmental parameters, activating the MTIST when Field modified microtiter immunospore trap pre-set conditions are met.

MTIST device is a portable, robust, and inexpensive system that can be used for multiple tests during a single sampling period, and it should be useful for monitoring airborne particulates and microorganisms in a range of environments. However, to improve retention on the base of the microtiter well of large or 'non sticky' microflora, further tests are required to determine suitable well coatings which will enhance the trapping efficiency of the MTIST.

Summary

Often, sampling or budget constraints limit the hypothesis testing approach to data assessment, and the amount of quantitative airborne data that can be gathered on suspect environments. The use of quantitative real-time PCR (QPCR) may greatly increase the hypothesis testing approach for airborne data by lifting many of the constraints of the present sampling schemes in use, as well as for its notable benefits of rapid speciation and instrumental traceability.

For airborne interpretations to be useful, data regarding airborne sample repeatability using QPCR-based sampling and analysis should be known. Specifically, spore-equivalent loading on air sample media may play a large role in sample repeatability, and may dictate target loading and thus sampling times industrial hygienists should consider

Further, the research study suggests that for quantitative Real time-PCR to be useful in airborne data evaluations, the industrial hygienists must factor in the sampling and analysis variability QPCR needs from the variability associated with the environments under investigation.