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BIOLOGICAL CONTAMINATION OF THE BUILDING ENVIRONMENT: SAMPLING AND ANALYSIS

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HISTORICAL BACKGROUND

Occupational-Related Illness Due to Microorganisms

Examination of the microbial environment began in the late 1600's when Antony van Leeuwenhoek observed microorganisms ("animalcules") in water. In the mid-eighteenth century Lazzaro Spallanzani concluded, after a lengthy series of experiments, that the "animalcules" observed in water were carried by the air. These organisms in the air and water were not considered potential disease agents until the work of Pasteur and Koch demonstrated that certain microbes could cause disease. The first pathogen purified and identified was Bacillus anthracis, a serious livestock pathogen and an agent of occupationally acquired illness.

Since the pioneering work of Pasteur and Koch a large number of occupational illnesses have been determined to have a microbiological basis. People involved in agricultural related occupations experienced large proportions of these illnesses. This is due to two factors; 1) agricultural workers have intimate contact with soil and 2) specialized environmental conditions on the farm permit high numbers of specific organisms to grow. For example, farmers often store large amounts of hay and grain in buildings that are relatively airtight. This storage condition leads to high humidity and temperature that results in the microbial decay of the organic material by thermophilic actinomycetes (Micropolyspora faeni, Thermoactinomyces spp.). These are the principal agents of farmer's lung disease. Farmer's lung disease has also occurred in mushroom workers exposed to compost dust[43]. The use of ventilators in barns and silos will reduce the temperature below the optimum for the growth of actinomycetes and therefore will reduce the incidence of farmer's lung.

Agricultural workers are also exposed to a wide variety of fungi (Aureobasidium, Aspergillus, Penicillium, Botrytis, Cladosporium, Alternaria spp.), many of which have been implicated in causing allergic reactions. Organic dust toxic syndrome has been linked to exposure to mold, bacteria, and bacterial endotoxins[18,19,63]. The agricultural worker is also exposed to zoonotic illnesses such as anthrax (which has almost been eliminated in the U.S.), Q-fever (which can infect both the worker and the surrounding community)[20], leptospirosis[82], and a host of other zoonotic agents.

Exposure to microorganisms in the industrial setting can also result in disease. Industrial workers that have contact with agricultural material have a risk of illness similar to that of the agricultural worker. Meat packers, tanners and textile workers are at risk from anthrax, although this is not a common disease in the developed world. The last reported case of anthrax in the U.S. was in 1992[73]. Previous to that a maintenance worker at a textile mill acquired anthrax in July 1987[9]. Slaughterhouse workers and workers in livestock related industries (hide, rendering, animal transport, woolsorters, etc.) are at increased risk of exposure to Coxiella burnetii, the agent of Q-fever[76]. Bakers can develop asthma due to exposure to flour and its contaminants such as fungi, mites and insects[73]. Cotton workers are at risk to byssinosis, which may be caused by exposure to bacterial endotoxins or Gram negative bacteria[8,14,38].

Workers in the biomedical field are at increased risk to a number of human and zoonotic infectious diseases including Hepatitis, Marburg, Simian B, Tuberculosis, Q-fever, Rabies, and Lassa Fever[31,39,57,66,86]. Workers in occupations which occur in rat infested environments (miners, sewer workers, dockhands) are at risk of exposure to leptospirosis[69,82]. Workers exposed to bacterial enzymes, such as those in the detergent industry, can have severe allergic reactions[49].

Office-Related Illness Due to Microorganisms

Office related illness due to microorganisms was not a problem until the mid to late 1950's. Office buildings, prior to this time, were similar to the home environment in that opening and closing windows controlled ventilation. When large-scale mechanical ventilation systems became practical the design of office buildings changed from relatively narrow spires with natural ventilation to large blocks with mechanical ventilation. Mechanical ventilation systems brought with them environmental choices, such as: the percentage of air that should be recirculated, the relative humidity, the method of humidification and the location of the intake and exhaust. It also brought in other variables such as frequency of maintenance and choices in the materials within the HVAC system. All of these factors affect the quality of the indoor environment[2,4,32,33,47,79]. Poor choices have resulted in office related illness in office workers.

The National Institute for Occupational Safety and Health (NIOSH) lists biological contamination as the third most serious indoor air health threat preceded by poor ventilation and chemical contaminants[87]. Poor ventilation, poor heating, lack of ventilation air-conditioning (HVAC) system maintenance, stagnant water in ventilation systems and relative humidity levels above the 60-70% range have all been implicated as causes of high indoor air concentrations of microbes[2,5,7,11,23,32,59,68,77,88]. It is important to realize that a small drop in temperature may result in a large change in relative humidity. At an absolute humidity of 12g/kg (weight of water contained per kilogram of air) a temperature change from 23.2°C to 21.5°C increases the relative humidity (RH) from 60% to about 75.5%. The environment changes from one that is hostile to fungal growth to one that is permissive to the growth of xerophilic fungi[42].

The common element in most cases of microbial contamination is the presence of water in cooling towers, humidifiers and water-damaged materials, and the subsequent dispersal of the organisms via the HVAC system. In some cases the location of the air intake was a direct cause of the microbial problem by bringing outdoor air over decaying plant matter or stagnant water.

DISEASE SPECTRUM OF BUILDING-RELATED ILLNESS

The disease spectrum of building-related illness is more limited than that of occupational-related illness. Generally, the illnesses are due to allergic reactions to the organisms rather than infection. The important exception to this pattern of allergic reaction is exposure to members of the Legionella genus. Legionella spp. were unrecognized until the famous outbreak of severe pneumonia in Philadelphia, Pennsylvania, during the American Legion convention of 1976. Since then, retrospective studies have shown that this disease agent has caused building-related illness for at least a decade prior to its identification[55,81]. It is also the agent responsible for the non-pneumonic illness known as Pontiac fever. In almost all cases of epidemic illness due to Legionella spp. the source of the organism has been water (cooling towers, potable hot water systems, showerheads, etc.)[6,15,22,29,30,45,46,56,61,85]. The common mode of transmission has been aerosolization of the organism.

Microbes, such as Thermoactinomyces sp., Flavobacterium sp., and endotoxins are thought to play a role in humidifier fever or humidifier lung. An inflammatory reaction in the alveoli and bronchioles results in flu-like symptoms such as fever, chills, headache, breathing difficulty and chest tightness[23,62,68,72,84].

Many building-related illnesses are allergic type reactions. The allergic reaction may occur on the skin, in the nose, airways or lungs. Effects on the tissue include dilation of the blood vessels, mucous secretion, cellular inflammation and contraction of smooth muscles lining the airways of the lung. Symptoms include runny or stuffy noses, sneezing, coughing, itching, skin rashes, malaise, headaches and breathing difficulty[10]. These symptoms have also been correlated with endotoxin exposure and $(1\rightarrow3)$-β-*D*-glucan (a cell wall component of fungi and some bacteria).[35,71]

The most serious building-related illness is hypersensitivity pneumonitis. This is an acute immune reaction in which the lung alveoli are filled with and damaged by inflammatory cells. This disease has been linked to exposure to a number of fungi and bacteria[27,43,75]. Continued exposure can result in irreversible pulmonary fibrosis with ensuing lung failure and death. The initial symptoms are similar to humidifier fever (flu-like) and may include breathlessness and nonproductive cough[37].

CHARACTERISTICS OF AIRBORNE MICROBES

A variety of organisms are responsible for building-related allergic reactions or infections (see table 1). The diversity of organisms in the environment often makes it difficult to isolate the causative agent or agents. For example, Legionella spp. do not grow on standard media, which is one of the reasons it was not discovered prior to 1976. Amoebae require plating onto a lawn of bacteria. Many organisms produce substances that are inhibitory to other organisms, and therefore, the slower growing species may not be isolated. While viruses can cause many human infections, they are not agents of building-related illnesses. A building can provide environmental niches for the multiplication of all of the agents listed in Table 1. Viruses cannot replicate outside of a living host, thus a building cannot provide an amplification site for them. Viruses can survive on environmental surfaces and poor personal hygiene can augment their transmission[1,34] but the building is not at fault. At most, a building's HVAC system may provide a means for them to more easily spread from person to person[11,17].

The concentration and types of organisms present depend upon the type of environment. Gram negative organisms are commonly found in feces and are common plant pathogens. Thus, if one samples in or near a sewage treatment facility, farms, grain or herb processing plants, Gram negative organisms can predominate[16,21,40,48,51,54,67]. Gram positive organisms are found on mammalian skin (especially cocci), soil, and some are plant pathogens. In an urban environment (both inside and outside of a building) where there is a high density of people and a low density of vegetation the vast majority of organisms are Gram positive cocci[53,83]. However, Significant increases in concentration of fungi and Gram negative organisms can be found when sampling near demolition of a building, improperly maintained humidifiers and water cooling towers. Soil and water are the primary reservoirs for molds but inside a building other sources and disseminators of molds (such as paper products, wallpaper paste, paint, wood, natural and synthetic fibers and leather) become important[3,43]. The cell walls of Gram negative bacteria inherently contain endotoxin. Thus, nonviable or even nonintact Gram negative bacteria may be a problem. It is important to note that there may be no relation between the number of culturable Gram negative bacteria in the air and the endotoxin concentration present[44].

The predominant organism(s) found in an environment are not necessarily the ones responsible for illness. The organisms that contaminate a product such as water or a filter will be those that may have one or more of the following characteristics:

- resistant to any biocidal or biostatic agent present;
- reproduce in a low nutrient environment;
- utilize a carbon compound that most other organisms cannot; and
- suppress the growth of potential competing organisms.

Organisms possessing these capabilities find a niche where they can outcompete their rivals. One of the major organisms responsible for the contamination of standing water and moist products is Pseudomonas spp. They are successful because they are resistant to biocidal and biostatic chemicals and can metabolize a wide variety of carbon compounds. Even though they can predominate in selected niches they are rarely a cause of building-related illness.

CONTROL METHODS

Office building HVAC systems should be designed and maintained to prevent illness or discomfort to the workers. Air intakes should not be located at or below ground level, over garbage, where they will entrain exhaust (from the building's HVAC furnace, bathroom exhaust or incinerator) or where they will entrain the plume from a water cooling tower.

Cool mist humidifiers have been implicated as a source of biological contamination[72,84]. Humidity should be supplied by steam rather than by mist. All too often, standing pools of water (whether in a cooling tower or a humidifier) become a source of microbiological contamination due to a deficient preventive maintenance program.

Filters can become contaminated with fungi and the passing air stream will spread the contamination throughout the ventilation system. For this reason, filter housings should be designed so that the filter can be easily checked and changed, thus encouraging (or at least not hindering) preventive maintenance.

Control of biological contamination in indoor environments may be achieved by:

- regulation of relative humidity levels (40-60%);
- proper design and maintenance of ventilation systems
- overall cleanliness; and
- periodic cleaning of all places where water is likely to collect.

Other possible methods of reducing microbial levels include:

- high efficiency particulate air (HEPA) filters;
- electrostatic air cleaners (EAC); and

- high intensity UV radiation in central supply ducts.
- However, these methods all have drawbacks. HEPA filters are expensive, EAC requires regular cleaning and UV lights produce ozone and require regular cleaning.

MICROBIAL AIR SAMPLING

Sampling Methods

There are numerous methods of air sampling for culturable microorganisms. These include:

1. Sedimentation—fall out plates
2. Sieve (cascade) impactors
3. Slit to agar samplers
4. Centrifugal samplers
5. Filtration & impingement methods
6. Precipitation methods

Sedimentation is a passive form of sampling. It is achieved by allowing "fall out" or gravity settling of particles onto agar plates. Air turbulence in the immediate vicinity has a great influence on the delivery of particles to the agar surfaces. The disadvantages of this method include the following:

- airborne particles that are too small to settle out quickly (droplet nuclei) may not be collected;
- long exposure times may result in drying of the media and subsequent poor growth; and
- the information gained is qualitative and not quantitative.

Advantages include the fact that it is inexpensive, little training is required to collect the samples and it is not equipment intensive.

The slit to agar, sieve, cascade and centrifugal samplers all employ centrifugal forces or a vacuum to impact particles onto agar surfaces. Collection by impaction makes it possible to enumerate the number of colony forming units (CFU) per unit volume of air. These evaluation methods rely on multiplication of microbial cells on a nutrient media and therefore cannot detect organisms unable to reproduce because the media or the growth environment is inappropriate or the cell was damaged by the stresses of aerosolization/collection. Also, if large concentrations of airborne particles are present they may be superimposed on one another upon collection and thus the actual bioburden will be undercounted. Other identification and quantification methods (such as the Rotorod Sampler) are required if allergic reactions to nonculturable cells are important.

Cascade samplers, such as the Andersen 2 and 6 stage, are useful for determining the size distribution of airborne particles. These units require a separate vacuum unit. The various stages allow the separation of different size particles. Overlap from stage to stage does occur.

In the slit to agar sampler, air is aspirated by a vacuum through a slit and the airborne particles are impacted onto a rotating agar surface. Due to this rotation, differences in the aerial bioburden over time can be observed. This unit is useful in demonstrating that ventilation, work practice techniques, clothing, activity, etc can affect the aerial bioburden level. The slit sampler may give lower counts versus cascade impactors and impingers. Thus, it may not be efficient for trapping small particles or useful in areas that have low numbers of culturable particles[24,28,50].

Centrifugal samplers, such as the Biotest, are convenient because they are lightweight and self-contained. Airborne particles are impacted on an agar strip as opposed to a plate. This unit is useful for collecting samples in locations that are difficult to monitor with some of the other methods, such as inside ductwork. Collection efficiency for the original Biotest model is a function of particle size (large particles are collected more efficiently than small). The newer Biotest Plus model has more uniform collection efficiency. Thus, without knowing the particle sizes, the sampling data for the original Biotest is only approximately quantitative.

Incubation of all agar media from these samplers must be carefully monitored to avoid dehydration of the media, excessive moisture leading to spreading of colonies, overgrowth of the media by fungi, and the merging of colonies.

Filtration employing solid filters, soluble materials and impingement into liquids can be used to collect culturable airborne particles. Liquids are most appropriate for large volume collection. The longer an impinger is operated the greater the loss of viable cells due to aeration. Collection directly into liquid, however, provides some protection for the microorganisms versus the other methods and allows initiation of damage repair caused by the rigors of aerosolization, aerosol residence time and collection[80]. Aggregates of cells, which would grow as a single colony on an impactor, are broken up in the impinger liquid. This makes it possible, through appropriate titrations, to enumerate the total culturable cell per volume of air. Calculation of inhaled dose, from this measurement of air concentration may be preferred for some disease agents, especially those for which a small number of organisms constitute an infectious dose.

Membrane filters have been found to detect approximately 79% of the colony forming units obtained with the Reyneirs air sampler[26]. Solid filter samplers may adversely influence the survival of airborne microorganisms due to impaction and dessication[52]. The use of gelatin air filters increases survival by decreasing the effect of dessication. Pore size must be carefully selected so those particles carrying bacteria and fungi are retained on the filter.

Collection of culturable airborne particles via precipitation is achieved by samplers that collect air and electrostatically or thermally precipitate particles onto a thin flowing film of collecting fluid.

The Rotorod, Burkard, and Samplair samplers are examples of quantitative samplers for the collection of pollen and fungal spores. They collect particles onto a sticky surface that can then be stained and examined microscopically for the presence of spores, hyphae and pollen. No viability determination is made and an experienced eye is often required to separate biological material from nonbiological debris. Since growth is not a criteria, information as to total numbers of spores and pollen can be obtained. This information can be quite useful in circumstances of allergic reactions. Microscopic examination, however, may be quite labor intensive.

All of the samplers give information as to what was in the air at the time of sampling. They do not predict what the concentration of organisms will be after the sampling or what it was prior to the sampling. The slit-to-agar, Burkard and Samplair can provide information on how the microbial population varies over the course of time. Fungi, like plants, release their conidia (spores) at certain times during the day; thus the fungal counts can vary greatly. For example, over the course of 22 hours, the hourly conidial (spore) level ranged from a low of 68.4 to a high of 410.7 per cubic meter of air sampled (unpublished studies by our laboratory).

The success of any method of microbiological monitoring depends on many factors. The capacity to form a colony depends on temperature, humidity, sampling medium, oxygen concentration, and competition from other collected organisms. The proper sampling device must be chosen for the intended task. For example, the typical range for culturable fungi in the outdoor air in the New England region varies from a winter low of about 50 colony forming units per cubic meter of air to a summer high of about 3000. Thus, in winter one would need a sampler that could draw in 300-500 liters of air in an acceptable time period while in summer one would only have to sample about 10-100 liters of air.

When to Sample

Ideally, microbial air sampling should only be undertaken when there is a medically validated reason to support that airborne mold or mycotoxin, bacteria or endotoxin is a probable cause of illness. Knowledge of the probable agent allows one to tailor the sampling protocol to maximize the recovery of the agent in question. For example, if one were dealing with an infectious agent, then culturable particle sampling using selective media would be initiated. If the diagnosis was allergic rhinitis then total mold spore and pollen counts would be important and total particle sampling would be performed. To sample without this information is often an exercise in futility. Outside of a cleanroom environment, there are always organisms present in the air. Thus, sampling will result in data but it is often meaningless information.

If there is obvious contamination of a surface, such as mold growing on damp carpeting, sampling is not necessary and remediation should be initiated immediately.

Sample Sites and Numbers

At a minimum, one needs to sample the test (problem) site, a control (nonproblem) site and an outdoor site (preferably near the air intake for the building). In a large indoor area, multiple samples are recommended (a sample for every 9-18 square meters). If the sample site is difficult to get to due to distance or restricted access, then it is recommended to perform duplicate samples to minimize the risk of an invalid sample requiring another trip to the sample location.

The microbial content of air is very heterogeneous and even taking two samples simultaneously, side by side may result in two very different counts (unpublished studies by our laboratory of 22 simultaneous samples yielded an average of 24% variation between the two samples [the range was 0.01% to 70%]).

Media Selection

Selection of growth media is of critical importance. There are selective, selective and differential, and general growth media. Selective media grow only a narrow range of organisms and are quite useful when one needs to know whether a certain organism is present. It is important to realize that selective media usually exert additional stress on the organisms and generally have lower recovery rates then nonselective media and that not all organisms will grow on a general growth media.

In most cases of building-related illness, no causal organism has been identified and thus one will have to use a general growth media in order to grow as many different organisms through a single sample as possible. For bacteria, tryptic soy with or without blood is a good general media. This media will not grow certain fastidious bacteria such as Mycobacteria or Legionella. For fungi there are a variety of general media such as Malt Extract, V8, Potato Dextrose, Rose Bengal, dichloran glycerol (DG-18), and Sabouraud-Dextrose. Of these, Rose Bengal and V8 gave significantly lower overall counts then the other media[12]. There are a number of different Rose Bengal formulas and not all underperform the other general media[60,74].

MICROBIAL AIR QUALITY STANDARDS

There are no formal standards concerning microbial air quality. Some studies utilizing tryptic soy agar and the 6-stage Andersen sampler have found a range of 10-100 colony forming units (CFU's)/M^3 in unoccupied rooms. Occupied rooms contained from 100 to 500 CFU/M^3 and occasionally the numbers were as high as 1200 CFU/M^3 [18,58,70,83]. However, there is no data relating these levels with illness.

It has been suggested that an excess of 1×10^3 CFU/M^3 indicates the need for investigation and possible remediation[58] but other experts feel that as little of 300 CFU/M^3 may warrant investigation[70]. Morey suggests that counts of microorganisms in stagnant water and in dusts of HVAC systems may be helpful. His preliminary conclusion is that bacteria/fungi levels in excess of 1×10^5 ml in stagnant water and fungi levels in excess of 1×10^6/g in dusts are indicative of excessive microbial contamination[58].

In the metropolitan Boston, Massachusetts area, outdoor fungal levels typically vary from about 50 CFU/M^3 in the winter months to 3000 CFU/M^3 during the summer months. In buildings with open windows and no amplification site(s), there is usually no difference between an indoor and an outdoor sample. In buildings with HVAC systems, the filters typically remove 60-80% of the airborne fungi and thus the indoor levels (assuming again no amplification site[s]) would range from 10 to 1200 CFU/M^3. Thus, if the indoor fungal level is $\geq$ 1000 CFU/M^3 and the outdoor count is $\geq$ 2500 CFU/M^3 does this represent a contamination problem or just low quality filtration? If the genera of fungi indoors reflect that on the outdoor sample, then one is not dealing with contamination.

There are no studies of dose/response relationships that describe the concentration of microorganisms that may impair health or cause occupant discomfort. It is difficult to assign a microbial permissible exposure limit (PEL) that applies to all situations, all of the time. Variable factors such as:

- different geographic locations and climates;
- types of environments (office vs. manufacturing);
- time of year;
- time of day;
- weather conditions (wind, humidity); and
- pollution,
- human activity

can affect the microbial population in the air and therefore influence air-sampling results[13,36,41,58,64,65,67,70]. Additionally, some organisms are more allergenic and some are more pathogenic. Thus, exposure to 100 Staphylococcus epidermidis per cubic meter of air would probably be harmless, however exposure to 100 Mycobacterium tuberculosis would probably be harmful.

INTERPRETING THE RESULTS/CASE STUDIES

The following case studies have been selected to help illustrate the points in the preceding sections.

Case 1

Occupants of several basement offices were experiencing pneumonia, respiratory problems, headaches, swollen lymph glands, irritated eyes and allergic type symptoms. The 2-stage Andersen sampler and Sabouraud Dextrose agar were used to take four-minute samples of four office areas. An outside control was taken. During the sampling, visible fungal growth was observed in the garbage area that was one room over from the office areas.

Two office areas had twice the number of fungal organisms per volume of air (1210 and 1157 CFU/M^3) than the outside air (618 CFU/M^3). The garbage area had four times the number of fungal organisms per volume of air (2509 CFU/M^3) than the outside air.

The predominant fungi in the office and garbage room air were the same, implicating the garbage room (with its visible fungal growth) as a possible source. The garbage room was damp and unventilated.

It was recommended that the garbage room be relocated and that the room be cleaned with a 5% chlorine bleach solution. Other measures taken included: replacement of several air handling systems, cleaning and disinfection of remaining air handling systems and relocation of air intakes to five feet above ground. The air intakes were originally located below ground level where leaf and other debris had accumulated. Pulling air from such areas is not ideal due to the high levels of microorganisms associated with soil and decaying plant matter. Also, the ventilation system was modified so that both outside and recirculated air was filtered. Previously, only the outside air had been filtered.

The offices were sampled again after all of the above changes were made. The results indicated that the offices had less fungal organisms per volume of air than the outside air. The above mentioned changes appear to have improved the air quality. The tenants exhibiting symptoms had left the building by the time the changes had been made. The remaining occupants did notice an improvement in the air quality.

Case 2

In another building, air sampling was requested following complaints of "bad" air. Three of the workers had seen their physicians who suspected that they were experiencing allergic reactions to something in the work environment. Air samples were taken for culturable fungi and total spore population since fungi are the most likely microbial cause of allergic reaction inside a building.

Culturable particle sampling yielded 6.3-18.8 Colony Forming Units (CFU)/M^3 inside the building, while the outside air had 125CFU/M^3. Based on this result, no microbial problem seemed apparent, however, the total spore counts were 5678/M^3 in the outside air versus 8796-11,530/M^3 indoors. Additionally, the indoor samples had 76-160 insect parts and 228-320 fiberglass fragments per cubic meter. This indicated that there might indeed be a problem in the air-handling unit (AHU).

When the AHU was examined it was found that the fiberglass insulation inside the mixing chambers had deteriorated. Samples of the fiberglass revealed numerous fungal spores, pollen, and some insects. Since it was probable that this was the source of the contamination, the insulation was removed and the area cleaned. Immediately after removal, air samples were taken. The counts had increased to 39,623 spores, 1,822 fiberglass fragments and 455 insect parts per cubic meter. Follow-up samples were taken after allowing the building to purge over a weekend. The spore level inside decreased dramatically to below that of the outside air and insect parts, fiberglass fragments were below detectable limits. The occupants of the building reported improvement in the air quality and relief from their allergic symptoms.

CONCLUSIONS

Since the discovery of microorganisms in the late 1600's, it has been determined that some microorganisms are responsible for occupational illnesses in agricultural, industrial and office environments. The disease spectrum ranges from allergic reactions to more serious illnesses such as Legionellosis and hypersensitivity pneumonitis.

Due to the ubiquitous nature of microorganisms in the air, sampling without knowledge of the agent causing illness may result in meaningless data. A medical opinion as to what organisms may be causing an illness provides direction in terms of sampling strategy. Some sampling strategies may be more appropriate for recovering certain organisms in certain environments. The sampling strategies differ in the data they provide (quantitative vs. qualitative), whether they can demonstrate aerial bioburden over time, whether they demonstrate the size distribution of particles and whether collection efficiency is a function of particle size. The type of organism and concentration are important factors to consider when selecting a sampling strategy.

There are no formal microbial air quality standards. It has been suggested that 1 x 10^3 CFU/M^3 or greater are levels which merit investigation. However, this would not be valid in a facility that has open windows during peak fungal growth months when the outdoor levels frequently exceed this level. In general, a mechanically ventilated uncontaminated space where the HVAC system has intact, inplace filters will have far fewer fungi then the outdoor air. Thus, indoor environments with HVAC systems and closed windows that have higher levels of fungi than outside should be investigated. Bacteria levels, on the other hand, are often higher in occupied spaces due to shedding of skin cells (and the bacteria on them) by the occupants. Hard and fast microbial permissible exposure limits will be difficult if not impossible to establish due to the effect of season, time of day, geographical location, weather and pollution, on the type and concentration of microorganisms in the air.

The most effective method of preventing office-related occupational illnesses due to microbes is proper HVAC design and maintenance. Air intakes must be located such that fresh, uncontaminated air is drawn in. Air supplies that are humidified should be humidified with steam rather than cool mists. Filters should be changed regularly. Places where water is likely to collect should be cleaned periodically. Water damaged materials, such as carpet or wallboard, should be replaced as soon as possible. All sources of moisture should be investigated and remediated as necessary.

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TABLE 1
Causative Agents of Building Related Illness and Allergies

Fungi	Bacteria	Amoebae	Algae
Alternaria	Micropolyspora faeni	Naegleria guberi	Chlorella
Aspergillus	Thermoactinomyces vulgaris	Acanthamoebae	
Aureobasidium	Thermoactinomyces candidus	Amoeba proteus[a]	
Cephalosporium	Flavobacterium sp.[b]	Enchinaboeba exudans[a]	
Cladosporium	Bacillus subtilis	Vahlkampfia sp.[a]	
Merulias lachrymans	Bacillus cereus[a]		
Penicillium	Legionella pneumophilia		

a) Probable identification

b) Endotoxin

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