

## **Sampling Limitations**

**Air sampling for mold spores and other bioaerosols has strong limitations.** The American Conference of Governmental Industrial Hygienists (ACGIH) in their 1999 booklet TLVs (Threshold Limit Values) and BEIs (Biological Exposure Indices) states: “Even when investigators work from testable hypotheses and well-formulated sampling plans, results from environmental bioaerosol monitoring may be inconclusive and possibly misleading.” This remains true today.

The ACGIH Bioaerosols Committee has developed guidelines for the assessment and control of bioaerosol exposures. These guidelines suggest five activities for evaluating bioaerosol exposures and for recommending control measures:

1. Visual inspection of the building.
2. Assessment of occupant symptoms.
3. Evaluation of building performance.
4. Monitoring of potential environmental sources.
5. Application of professional judgement.

The information collected through air monitoring is only one of the factors that should be considered when selecting the approach to use when bioaerosol exposures are suspected. Reliance on the “numbers” alone may result in inappropriate decision-making due to the limitations of this monitoring. These limitations exist because:

- a. Different methods of sample collection and analysis may result in different estimates of mold concentrations. Different sample media (agar) results in the growth of different quantities and different types of mold.
- b. The actual cause of health effects from mold is not being quantified when mold spore counts (how much mold grows on a culture plate) are made. Actual antigens (the agents causing an allergic reaction) are not quantified. The concentration of “culturable (viable) fungi” (that which will grow on a culture plate) is used to represent the concentration of antigens. Antigens, however, may be present in an environment as particles from a spore coating which will not grow (are not “culturable” or non-viable) on an agar plate. **This results in an underestimation of exposure.**

Mycotoxins may be the source of additional health effects from mold. The concentration of “culturable fungi” does not quantify the concentration of these chemicals in the environment.

c. The most commonly used sampling devices are those that direct air flow onto a culture plate. This method, while minimizing the loss of culturable fungi through spore damage or drying, has two strong limitations. The sampling device is too large to collect personal breathing zone samples requiring "area" samples. These "area" samples may not accurately reflect human exposure. Second, the samples are taken for a short period of time (minutes) to prevent overgrowth on the culture plate. Some fungi release spores as "concentration bursts" that may not be detected in the short samples. It is suspected that these bursts may produce health effects.

**This consultant is concerned that, overall, these limitations result in an UNDERESTIMATION of exposure.**

### **Minimizing the Sampling Limitations**

The sampling protocol used by Sharon J. Bessa & Associates, Inc. is designed to minimize the limitations:

1. Two sample sets are taken at each collection site, at least two hours apart. This provides at least two "snapshots" of the mold spore concentrations.
2. Two or three different agars are used for each sample set. This provides 4 to 6 sample plates at each collection site.
3. The samples are taken in an "aggressive" manner. Mold spores are particles that settle on horizontal surfaces after being released. Brushing or raking the carpet, moving papers or books, etc. makes spores airborne while the sample is being taken.
4. "Control" samples are taken in locations where there is no known history or evidence of water damage or mold growth. These samples provide information on the background level of mold contamination in a building. Reference may also be made to this consultant's data base for typical background levels.
5. Samples may also be taken for non-viable spores or particles.

### **Quality Control**

1. The sampling pump is calibrated before and after each sample day.
2. Cross contamination is minimized by establishing a "clean" and a "contaminated" work space for handling the sampling equipment. The equipment is disinfected between each sample set and each plate is sealed in a plastic bag after the sample is taken.
3. Blanks (unsampled agar plates) are sent for analysis from each location.

4. Samples are kept in a cooler prior to being delivered to the laboratory by the consultant; they are not shipped nor sent by courier.

### **"Acceptable" Limits**

Acceptable limits for mold sample concentrations (air or bulk samples) have not been established by the Occupational Safety and Health Administration, the Environmental Protection Agency, the National Institute of Occupational Safety and Health or the State of Wisconsin. There are no current limits established by the American Conference of Governmental Industrial Hygienists (ACGIH) for either total mold spore counts or for specific mold genera or species. ACGIH summarizes the reasons for this lack of established limits:

1. Health effects vary from one mold genera to another.
2. The type and severity of health effect varies with individual susceptibility. While the most common response to mold exposure is an allergic reaction, recent studies have suggest an association between mold exposure and a variety of systemic health effects.
3. Information relating mold concentrations to specific health effects is insufficient to establish a dose – response relationship. The absence of meaningful epidemiological data is due to the limitations of sampling.

**"Acceptable" concentrations are provided by this consultant based only on a comparison to control areas or locations where there is no known history or evidence of water intrusion or occupant concerns about their symptoms. Acceptable concentrations in relation to avoiding health effects have not been established and are not provided.**

### **How the Samples are Taken**

The sampling device (Andersen 2-stage) consists of two metal filter plates and a pump. A plate of culture media (agar) is placed inside the device below the filter plates. Air is drawn by the pump through the filter plates and mold spores are deposited onto the agar. The air is collected for one to three minutes for a total volume of 28.3 liters per minute.

The plates are removed from the sampler, covered and sealed in a plastic bag. They are taken to the laboratory where they are incubated at 25 degrees Centigrade for 5 to 7 days. The total number of colonies are counted on each plate and the plate is viewed under the microscope to identify the mold by genera (in some cases, by species.)

Results are presented as a concentration: Total Colony Forming Units per Cubic Meter of Air. Although a cubic meter of air is not collected (only 28.3 liters per minute are collected; there are 1000 liters in a cubic meter), the results are presented as if a cubic meter had been collected to allow for comparison of samples when different volumes are used. One colony on a culture plate from a sample collected with 28.3 liters of air is equal to 35 colony forming units per cubic meter of air. (28.3 liters divided by 1000 liters [a cubic meter] is 0.0283. 1.0 colony divided by 0.0283 cubic meters equals 35 colony forming units per cubic meters of air.)

### **Reviewing the Sampling Results**

There is no requirement to perform air sampling and there is unlikely to be such a requirement in the near future. Some organizations may wish to forego the expense of sampling and spend their money on remediation. This is acceptable. However, despite the strong limitations, air sampling does provide some quantification of current conditions. These results can be compared to the concentration of mold detected after remediation. This may be important where mold is suspected but is not visible.

Building environments are not sterile and detection of mold spores is expected. Both the concentration and the type of mold detected are important factors to consider in interpreting the results and making recommendations for remediation. The following guidelines for interpreting the sampling results are compiled from three sources:

1. American Conference of Governmental Industrial Hygienists: Bioaerosols Assessment and Control Cincinnati, 1999.
2. American Industrial Hygiene Association: Field Guide for the Determination of Biological Contaminants in Environmental Samples Fairfax, 1996.
3. This consultant's experience and professional judgment.

The following factors are considered in interpreting mold sample results:

1. Total Count of Colony Forming Units per Cubic Meter of Air  
This factor shows the greatest variation between the two sample sets taken at each location. It is not unusual to find variations greater than ten-fold between the two sample sets. Typically, there is less variation between the concentrations of mold growing on different agar than between the two sets of samples taken at least two hours apart. This is probably a reflection of the "concentration bursts" or the episodic release of mold spores by the fungus.

Total Count Comparisons are made in two ways:

a. Between samples taken indoors and outdoors. When a mold problem does not exist, the spore concentration in the building should be less than the concentration detected in outdoor samples. However, comparison to outdoor samples must be done carefully - this is only a very crude way to ascertain what is acceptable in a building. The limitations of this comparison are illustrated in Example #1 below.

b. Between samples taken in suspect areas and control areas.

The mold spore concentration in most control areas is typically less than or equal to 247 - 300 colony forming units per cubic meter of air (CFU/m<sup>3</sup>) in viable samples and less than 3000 CFU/m<sup>3</sup> in non-viable samples. Therefore, when this concentration is exceeded, this may indicate a source of mold at a sample location. Comparison to controls areas within the same building are a more reliable indicator of a problem than comparison to outdoor air concentrations.

"Acceptable" limits are established as a guideline for building inspection and remediation only. "Acceptable" limits for health effects have not been established.

## 2. Genera and Species

Mold is identified by microscopically examining the "fruiting bodies" or stalks of the mold where spores are produced and released. In most cases, it is possible to identify the mold by genus (plural is genera). The genus is capitalized and in italics: *Cladosporium*, *Penicillium*, *Alternaria*. Some mold can also be identified microscopically to the species level. Examples are *Aspergillus versicolor*, *Stachybotrys atra*. If the mold does not produce fruiting bodies these are identified only as "Non-sporulating fungi".

The following mold genera are found in most samples taken outdoors in Wisconsin: *Cladosporium*, *Penicillium*, *Alternaria*, *Epicoccum* and *Aspergillus*. These are also commonly detected in indoor samples. In interpreting sample results the dominant genera/species in the indoor samples is compared to the dominant genera/species found in the outdoor samples. An indoor mold source is suspected when the dominant type of mold in indoor samples is not the same as the dominant type detected in outdoor samples.

3. Most mold comes from soil and leaf decay and the primary health effect is allergic reaction. Some mold causes greater concern and caution when identified in indoor samples. These molds are known to produce mycotoxins or chemicals that have been associated with symptoms other than allergic reaction. Examples of these include:

*Stachybotrys atra* or *chartarum*  
*Aspergillus versicolor*  
*Aspergillus fumigatus*  
*Fusarium monilitiform*

It is important to review sampling results without undue emphasis on any single factor or any single sample. Two examples illustrate the importance of considering all factors and applying professional judgment:

Example #1 Total colony counts taken indoors may be the same as outdoor air. This may indicate a problem, but, if the building has a lot of natural ventilation or if the samples were taken in an area where there was a lot of foot traffic (mold spores enter the building by ventilation as well as being tracked in on shoes and clothing), this may simply indicate contamination from the outdoors. Outdoor air sample concentrations vary with the season and the weather within each season. Obviously, outdoor air samples are not taken in the winter months or when it is raining. These and other factors that affect outdoor air concentrations must be considered in making this comparison with indoor concentrations.

Example #2 One colony of *Aspergillus versicolor* on a culture plate (this is equal to 35 colony forming units per cubic meter of air) does not necessarily indicate an indoor source. However, if there are six colonies and this is matched with a history of flooding with dry wall that remained wet over 48 hours, this may indicate an indoor source for mold.

Each of the sample location results should be interpreted using the above four items along with the visual inspection of the building, assessment of occupant symptoms, evaluation of current and historical building performance. This, along with professional judgement, will result in a reasonable interpretation of the data.

### **Remediation of the Mold**

Remediation or removal of mold growth is necessary to:

1. Halt exposure to excessive mold spore or mycotoxin concentrations.
2. Prevent continued growth of the mold.
3. Prevent or minimize deterioration of building materials.

Mold growth is very difficult to control once it has grown in building materials and it is best to use a professional service company to perform the remediation.. Mold has roots (some mold has longer, more invasive roots than other molds) and, in some cases, it is necessary to remove the building material itself to remove the mold. Mold spores are present in most materials such as fiberglass insulation. In some cases, a bulk sample of the material is taken to quantify the concentration of mold spores per square inch. Many materials contain mold spores in hundreds of colony forming units per square inch. When materials are excessively contaminated with mold spores the concentration is in the thousands per square inch.

The presence of an excessive concentration of mold spores may cause health effects in building occupants. Building materials must be kept from becoming too damp or wet to prevent active mold growth. Measuring the moisture content of a building material may provide an indication of whether the material should be removed. The use of a high efficiency or microfiltration vacuum cleaner on carpets and other surfaces is important in reducing the spore concentration when a building has been contaminated. This should always be done prior to or in place of any type of wet extraction. If the mold growth is extensive, it may be necessary to isolate the area and remove the mold and moldy materials using work practices that are similar to those used in asbestos abatement procedures. There is never a guarantee that mold growth will not recur in a building that has been contaminated. Remediation professionals will want baseline air sampling and follow-up sampling after clean up to objectively measure their progress or success in reducing mold spore counts.