CHAPTER 13, SECTION 2

MOLD AND OTHER BIOLOGICAL CONTAMINANT ASSESSMENTS: INVESTIGATING, SAMPLING, AND INTERPRETING RESULTS

INTRODUCTION

Microbes … microbiologicals … bioaerosols … biological contaminants - all terms for the broad category of airborne particles that are living or have biological origins and are generally part of our natural ambient environment. This includes fungi, bacteria, viruses, protozoans, pollen, animal dander, insect parts and feces, and human skin scales. While these are all important in indoor environmental quality (IEQ) assessments, by far the most widely suspected, sampled, and publicized members of this group are the fungi. This section will discuss primarily mold assessments, but will also cover some other biological contaminants as well.

The number of requests for mold investigations has been steadily climbing, not only in the Navy but nationwide. There are no regulations or standards for mold, so it is often difficult for occupational health professionals to interpret data. Further, most of the underlying reasons for mold contamination originate with building construction or maintenance problems, so it may be difficult for the industrial hygienist to effect the changes needed to resolve occupant complaints.

"RULES" FOR MOLD AND OTHER BIOLOGICAL CONTAMINANT ASSESSMENTS

The following tenets are the foundation for all biological contamination investigations:

1. **Prevention** is the best way to keep biological contamination from becoming an issue. The key elements of prevention are **timely maintenance** and **prompt repair** of facilities and general **moisture control**.

2. Investigations are a **team effort**, requiring the assistance and cooperation of industrial hygiene (IH), occupational medicine, preventive medicine, safety, occupants, labor representatives (if applicable), facilities and maintenance personnel, housing or office managers, and command public affairs officers.

3. Open, honest **communication** is vital between the personnel conducting the investigation, occupants, and management. At least one team member should be trained in risk communication.

4. As it is typically the result of excess moisture on materials indoors, if biological contamination such as mold is found or suspected, **immediate action** is required to identify and fix the water intrusion source, dry the area, and clean or discard contaminated items. The goal is to minimize the health risk for occupants. While healthy individuals are seldom at risk from mold exposures, there is increased concern for those who are very young, old, debilitated, immunocompromised by other diseases or severely allergic to mold.

INVESTIGATOR PROTECTION

1. Do not disturb contaminated areas or aerosolize biological material.

2. Do not touch visibly contaminated areas with your bare hands. If you do, wash thoroughly with soap and water as soon as possible.

3. If you have to perform destructive sampling in an area (e.g., remove a section of wallboard to access the wall cavity) or disturb a substrate that you suspect is contaminated, use appropriate personal protective equipment (PPE) and lightly spray surfaces with amended water (contains a surfactant) to minimize the possibility of aerosolizing spores.

4. Recommended PPE for those assessing and/or sampling contaminated areas includes disposable gloves; disposable coveralls; goggles; NIOSH approved half face N-95 respirator (disposable is OK). PPE for remediation projects is discussed in the section 13.3.

COMMUNICATION

1. The principles and techniques learned in risk communication training are essential for biological contamination projects. Residents in Navy housing and office employees are sure to have seen some of the nationally televised programs about grossly mold contaminated houses and buildings with occupants who claim they can no longer function normally. There are hundreds of web sites about mold contamination, and most people have read articles about schools or homes contaminated with "toxic molds."

2. The more informed employees are about what is happening, the less likely they are to be fearful. Tell them in simple terms what has been found and what you will do to correct the situation. If remediation is required, tell employees what will be done, give them the remediation schedule, and explain how they may be affected (e.g., temporary relocation; control measures; testing). Provide medical support from the cognizant clinic for those with medical concerns or those who develop symptoms they believe to be associated with the contamination. Answer questions honestly and calmly, provide facts sheets tailored to the situation, and provide a contact list for medical and IH issues. Involving employees in the process gives them a stake in the successful outcome.

3. When mold or other biological contaminants are found, it is important to make sure that occupants are fully informed about what will be done to correct the building problems. Make sure that points of contact are identified by name so occupants can call if they have IH, medical, or remediation concerns. Section 13.5 contains a detailed discussion of risk communication.

ASSESSMENT STRATEGY

1. **Visual Inspection**

   a. Always conduct a thorough visual inspection first, evaluating the building with a critical eye toward potential problem sources. Look for signs of water damage on the ceiling, walls, and floors. Inspect the ventilation system (air handling unit, ducts, fresh air intake location, dampers). Locate odor sources, and look for possible chemical and biological contaminant sources or reservoirs.

   b. Likely sources or areas to check for water leaks include the roof; loose or damaged soffitts and gutters; chimneys; through-roof pipes or vents; improperly sloped drains; improperly vented appliances, uncontrolled humidity (e.g., moisture condensing on surfaces); improperly installed vapor/moisture barriers or surface finishes (e.g., exterior insulation and finish system (EIFS) or unsealed stucco).

   c. Simple tests may be helpful to determine the extent of damage or contamination. For example, a boroscope can be used to check the condition of ventilation ducts. A moisture meter can quickly identify wet building materials. Assessing indoor thermal conditions (temperature and relative humidity [see ASHRAE 55-2004 or Section 13.1 for acceptable ranges]) can also help identify areas where mold reservoirs are likely.

   d. If visible mold is found, locate the source of water and repair to prevent additional water damage. Proceed with cleanup and remediation procedures in Section 13.3.

      If mold is not found during the visual inspection, but the team believes there is biological contamination in the building (because of odors, visible water damage, employee illnesses, etc...), take additional investigative steps.

2. **Additional Investigation**

   a. Review building plans and check maintenance and preventive maintenance schedules for possible relations between mechanical component locations, maintenance procedures, and complaints.

   b. Talk with employees about their complaints and symptoms, especially anything different or unusual in the building that they may have noticed or whether they detect any pattern in their symptoms or with problems in the building.

   c. Check the building's relation to nearby industrial operations for potential pathways that might introduce contaminants.

   d. Investigate possible hidden mold reservoirs. This may require destructive procedures, such as removing wall coverings, wallboard, carpet or floor covering. Consider that there might be concealed growth behind walls, paneling or wallpaper, under floors, in electrical or plumbing chases, or in ducts.

Collecting screening air samples can help locate the general area of unseen mold reservoirs. Consider sampling for viable or non viable-bioaerosols (fungal spores), glucans, ergosterol, total microbial volatile organic compounds (MVOCs) or mycotoxins, etc...).

If mold is found, proceed with remediation per Section 13.3. Locate and fix the water source to prevent further intrusion.

e. Investigate and test for other possible biological contaminants. This might include sampling for bacteria, endotoxins, allergens (mites, dander, etc…) or checking on neurosensory factors (e.g., visual or perception disruptors).

SAMPLING STRATEGY

DO NOT collect samples without a sampling plan that details how and when samples will be taken, collection requirements for each type of sample, what criteria will be used to interpret results, and what benefits you expect from sampling, i.e., what question(s) will be answered and what actions will result.

ALWAYS consult the analytical laboratory before sampling to ensure sample collection and shipping are done per the laboratory's requirements and that results will meet your expectations.

1. **When to Sample**

   a. **The rule of thumb in biological contamination investigations is Do Not Sample when visible mold is present. Regardless of the mold identified or the number of spores, it does not change the requirement to stop the water intrusion and clean up the contamination**.

      (1) This is probably one of the biggest challenges during the investigation, since sampling is a natural action for industrial hygienists and a normal expectation from occupants.

   b. If you cannot collect a sufficient number of samples to fully characterize the site (i.e., because of funding constraints or insufficient sampling media), it is probably best not to collect any samples. Inadequate sample data usually lead to misleading or confusing results.

      (1) The investigation team should be guided by their collective expertise in deciding whether or not sampling is indicated. The following are some situations in which sampling is indicated:

         (a) If an occupant has been diagnosed with a disease that is caused by a specific biological contaminant or the physician suspects an association between symptoms and biological contaminant in the workplace, the physician may request confirmation of the presence of the causative agent.

(b) If remediation is required, pre- and post-remediation sampling can be used to verify success of the decontamination. Surface samples are especially useful.

(c) If the investigation team suspects biological contamination but cannot find evidence such as visible mold, air sampling may help to verify or locate the contamination. In such cases, air sampling could include testing for bioaerosols (viable and/or non-viable such as fungi or bacteria), mycotoxins, MVOCs, endotoxins and/or allergens.

(d) If litigation is underway or anticipated.

(e) If the ventilation system was cleaned/ remediated because of biological contamination (verified by visual or bulk/swab samples), use air sampling to determine if the areas supplied by the system are ready to reoccupy, that is, the ventilation system is not distributing bioaerosols.

c. Because you may be sampling biological contaminants such as mold whose presence depends on environmental conditions (heat, light, water availability), carefully consider ambient weather conditions.

(1) Rain can "wash" the air clean of many spore types, such that sampling on rainy, foggy, or very humid days can result in low outdoor counts or species distributions that are significantly different from those on warm, sunny days. In general, levels of ascospores and basidiospores will be higher during rainy weather.

(2) Sampling when there are strong winds can result in outside spore counts that are significantly higher than on non-windy days. In addition, high outdoor spore counts may mask small to moderate indoor mold problems since interpretation is dependent in part on ratios of indoor to outdoor spore counts.

(3) Compensate for ambient conditions by adjusting your sampling schedule if possible. At least be aware that outside samples may not represent normal conditions so that you do not misinterpret results.

2. **Where To Sample**

a. Complaint/problem area – Use complaint patterns, symptom descriptions, and visual indications to guide you in choosing sample locations. You may need a sampling array within a single office, on an entire floor of the building, or throughout the building to get results that are representative. Preliminary or screening samples may help target the areas that require further characterization.

b. Non-complaint area – Non-complaint area results serve as controls to compare with complaint area results.

c. Outside – Outside samples must be taken at the same time as indoor samples so that the types and quantities of ambient flora can be compared with those in the building. Ideally, at least one outside sample is collected at the fresh air intake that supplies the inside area being sampled.

3. **Number of Samples**. There is no formula to determine how many samples you need to characterize adequately a complaint area. Further, statistical validity considerations cannot be used because of the difficulty in predicting the environmental variability. The American Industrial Hygiene Association (AIHA) Field Guide for the Determination of Biological Contaminants in Environmental Samples gives this guidance:

   a. The number of samples depends on the size and organization of the space being investigated.

   b. Sample as many locations within the area of study, control locations, and outdoors as is practically and economically feasible.

   c. When possible, take duplicate side-by-side samples. According to Chapter 3 of the AIHA Field Guide, "duplicate side-by-side sampling is considered adequate to define the mean and the random sampling and analysis error given the high temporal and spatial variability of bioaerosol concentrations in air." …. "Acceptability of the agreement between side-by-side duplicate samples must be determined by the investigator based on the intended use of the data."

   d. Investigate temporal variations by sampling at least two time periods during the day, preferably separated by a long interval, e.g., morning and late afternoon. Sample on different days or during different seasons if daily/seasonal variations appear to influence conditions.

4. **Choosing What to Sample**. Appendix 13.2-A summarizes various sampling methods based on analyte. Additionally, Chapter 13 Section 6 provides various information resources. However, not all vendors, products and resources available may be included. Appendix 13.2-A and Chapter 13 Section 6 are meant as overviews of various sampling methods and resources to assist Navy Industrial Hygienists. They do not imply endorsement by the Department of Defense, the Navy, or the Navy and Marine Corps Public Health Center of particular methods, products, laboratories or vendors, etc…, nor does it mean that these resources' content have been validated. **Consult the laboratory that you will be using to perform the analysis for specific sampling methods, procedures and equipment.**

   a. Fungi, Bacteria, Allergens and Other General Biological Contaminants

      (1) Viable (culturable) vs. Non-viable (non-culturable) samples

(a) Viable samples (fungi, bacteria, etc…) are typically collected on nutrient agar initially, or can be collected in/on inert media (impinger liquids, filters, vials, bulk collectors, swabs) and prepared for culture at the laboratory. Samples are incubated for several days to allow cell growth and replication into visible colonies. The entire colony is used for the identification, allowing the laboratory to make a more exacting identification of certain types.

Culturable samples tend to underestimate the number of total cells present, since only viable organisms will grow. In addition, of the viable fungi or bacteria that impact onto the agar during sampling, only a percentage of those will actually grow during incubation.

Further, remember that some organisms require specific nutrients or growing conditions. If these are not present, the organism will grow very slowly or not at all. (For example, Stachybotrys requires cellulose. If you use Malt Extract Agar (MEA) for sampling and the report shows no Stachybotrys, this means that (1) there really was no Stachybotrys in the sampled area; or (2) Stachybotrys was present but MEA did not support its growth. A better alternative, in this case, is to collect a non-viable sample since the spores are very distinctive and can easily be identified by direct microscopic examination.) For sampling any organisms, contact the laboratory to determine the nutrient agar of choice for collecting viable samples.

Also, some sampling methods work better than others for viable samples depending on what you wish to find. For example, the recovery of viable fungi is typically much better than the recovery of viable bacteria using filtration methods with standard filters. This is due to bacterial cell desiccation. You can increase viable bacteria recovery using filtration methods by using a gelatin filter.

(b) Non-viable samples (fungi, pollen, mycelia, fiber, skin cells, fiberglass, etc…) can be collected in/on a variety of media (impinger liquids, sticky tape/slides, filters, vials, bulk collectors, swabs) and are examined directly under a microscope to identify and count spores (genus identification) and other particulates (e.g., mycelial fragments, fibers, skin cells) based on morphological features.

Some molds, such as Aspergillus and Penicillium, cannot be distinguished by their spores alone, so they are reported as a group (e.g., Aspergillus/ Penicillium group; Drechslera/Bipolaris group; or Smuts/Periconia/Myxomycetes.

(2) Allergens - Allergens can be viable and/or non-viable biological contaminants that produce allergic reactions in sensitive individuals. Typical allergens that are analyzed are produced by dogs, cats, rats, mice, cockroaches, dust mites and some fungi. Allergens can be collected in/on a variety of media (impinger liquids, filters, bulk collectors, swabs) and are analyzed by fluorescent immunoassay,

enzyme immunoassay (ELISA), radioimmunoassay (RAST) or Multiplex Array for Indoor Allergens (MARIA). These analyses are based on the specificity of an antigen-antibody reaction. Cross-reactivity can occur.

(3) Fungi and Bacteria (Genetic Identification) - Some fungi and bacteria can now be identified by Polymerase Chain Reaction (PCR) analysis. These samples can be collected in/on a variety of media (impinger liquids, filters, bulk collectors, swabs). This analysis provides genetic confirmation of certain fungal and bacterial species using species-specific DNA probes or primers. PCR is specific, but the technology is limited to the species probes available for fungal and bacterial confirmation. Consult the individual laboratories that offer PCR to determine what fungi and bacteria are in their detection panels.

(4) Fungal and Bacterial Metabolites and Cell Components

(a) β – Glucans and Ergosterol

(i) Both (1→3)-β-D-glucan (glucans) and ergosterol are fungal cell wall components of filamentous fungi, which includes most saprophytes. Sampling will detect these components in both living and dead spores. These compounds have been sampled successfully as chemical markers to show that such fungi are present. There is little data comparing the number or mass of spores to chemical marker concentrations.

(ii) Glucans are collected by filtration, and are extracted and analyzed using a limulus amoebocyte lysate (LAL) or ELISA.

(iii) Ergosterol is also collected by filtration, and is extracted and analyzed high performance liquid chromatography (HPLC), gas chromatography (GC), or GC with mass spectrophotometry (MS). Ergosterol is reasonably stable in spores.

(b) Endotoxins - Endotoxins are found in the cell walls of gram negative bacteria. Made of lipopolysaccharides, they can elicit health effects in susceptible individuals whether the bacteria is viable or not. The most common exposure routes are inhalation and ingestion. Gram negative bacteria are most often associated with water, sewage, humidifiers, and gray/black water contamination. Endotoxins are collected on endotoxins-free filter cassettes or by bulk sampling. The samples must be collected carefully to ensure there is no contamination. Analysis can be done by LAL, kinetic chromogenic assay or turbidimetric assay.

(c) Extracellular Polysaccharides (EPS) - EPSs are stable carbohydrates that are produced during fungal growth. EPSs have antigenic specificity, usually at the genus level, and are analyzed by ELISA immunoassay.

(d) Fatty Acids - Fatty acids are bacterial cell wall components. Analysis uses GC, or GC-MS to determine the fatty acid profile, then compares results to a reference database using statistical pattern recognition software.

(e) Microbial Volatile Organic Compounds (MVOCs) - MVOCs are produced by fungi and bacteria that are metabolically active. They are also responsible for many of the musty odors associated with molds. If you 'smell mold' but cannot see it, MVOC sampling may help to locate the fungal reservoir. MVOCs are collected using sorbent tubes or summa canisters and analyzed by GC, or GC/MS.

(f) Mycotoxins - Fungi are primarily saprophytic, that is, they use nonliving organic material as the nutrient source for growth and reproduction. During the digestion process, fungi secrete enzymes to help break down complex compounds into simpler ones that can be taken up and digested. The by-products of digestion are classified as primary or secondary metabolites. Primary metabolites are produced from cellulose and other compounds that are used by the fungus for energy, growth, and reproduction. Secondary metabolites are natural by-products that are not necessary for growth and are usually derived from precursors formed during primary metabolism. One type of secondary metabolite is mycotoxins. Only some species of molds produce mycotoxins and they usually only produce them some of the time. Mycotoxins are thought to give the fungi a competitive edge against other microorganisms, including other fungi. Whether a toxigenic fungus actually produces mycotoxins appears to depend on environmental conditions, including temperature, moisture, growth substrate, aeration, pH, and existing stress factors. Some of the mycotoxins most commonly associated with mold contamination in buildings are briefly described in Appendix 13.2-B.

Mycotoxins accumulate in spores, mycelium, and growth substrates. Consequently, they can be inhaled (when spores or substrates are disturbed and aerosolized), ingested (consuming toxin-containing spores when eating, drinking or smoking in a contaminated area), or absorbed through the skin (e.g., when handling contaminated materials). Mycotoxins are generally nonvolatile and so are not inhaled directly (i.e., they do not off-gas).

Because air sampling for mycotoxins has limitations, bulk or surface samples are usually best. Mycotoxin samples can be collected in/on a variety of media (filters, bulk collectors, swabs) and are analyzed by liquid chromatography (LC) with MS, HPLC, GC-MS, solution fluorimetry or ELISA immunoassay.

5. **Choosing the Appropriate Type of Sample (Air, Bulk or Surface) and Sampling Methods.** Before taking a sample, think about why you need the result and what you want the results to tell you. For example, if you are trying to determine if an area is contaminated or if what you see is really mold, a swab or bulk sample may be sufficient.

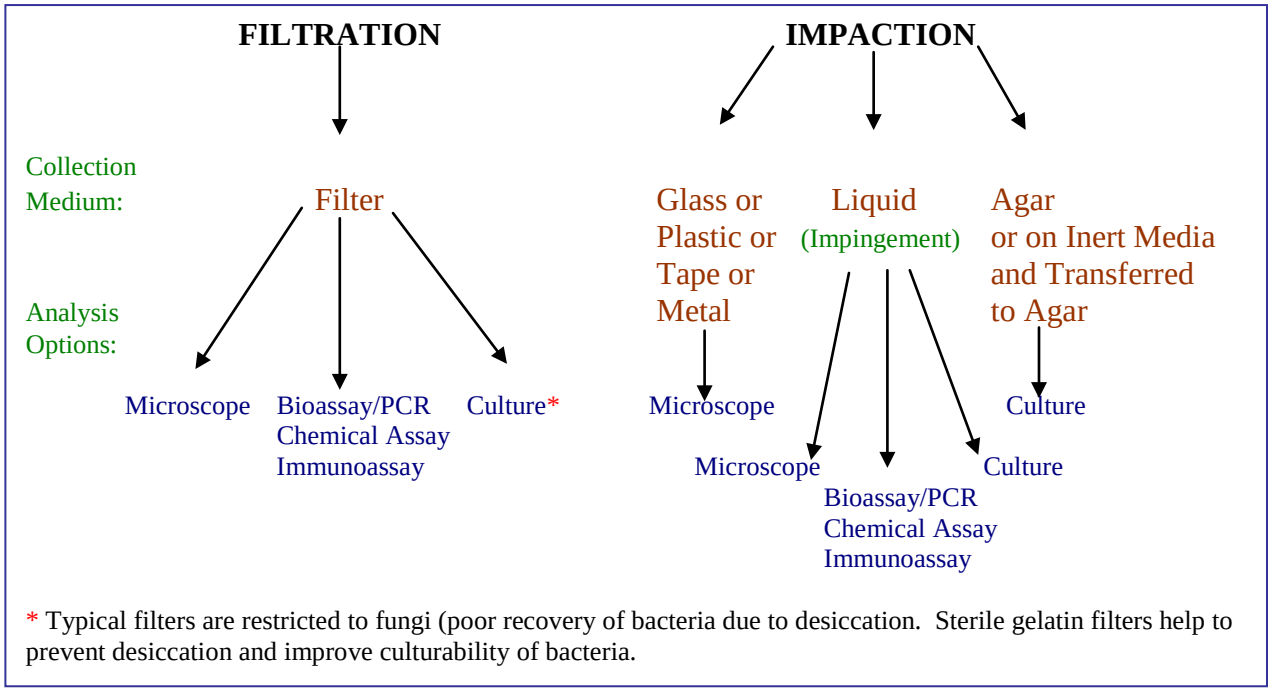
Consult the laboratory that you will be using to perform the analysis for specific sampling methods, procedures and equipment.

a. Air samples - Air sampling is the most common collection method for bioaerosols. A pump is used to draw in air and deposit the particulate onto a collection medium. Various air sampling methods can be used for microscopic analysis, culturing techniques, and/or specialized testing. Each kind of air sample has its benefits and disadvantages, depending on the media used and the collection and analytical method chosen. Regardless, air sample results for molds and other bioaerosols are subject to false negative results. That is, there may be contamination present even when results indicate otherwise.

(1) Bioaerosol collection principles for air sampling - In general, collecting bioaerosols involves either filtration or impaction. Figure 13.2-1 shows the collection methods and the possible analyses that can be performed using each.

(a) Filtration - Bioaerosol is collected on a filter as air passes through it. Filter media can have different diameters, pore sizes, and composition, so consult the laboratory before sampling.

(b) Impaction – Bioaerosol is impacted onto a collection media such as glass or plastic slides (may contain collection strip of agar, grease, adhesive, or tape), glass vials, metal impactors, liquid, or agar plates. Impaction into a liquid medium is also called impingement.



* Typical filters are restricted to fungi (poor recovery of bacteria due to desiccation. Sterile gelatin filters help to prevent desiccation and improve culturability of bacteria.

Figure 13.2-1. Bioaerosol collection methods.

(2) Why mold size is important - Particle collection efficiency is driven by the size of the particle you want to collect. With spores, this can make the difference in whether a negative result means there really is no mold present – or that the mold is there but you did not collect it. For example, Cladosporium cladosporoides is around 2 µm in size. If you suspect that you have Cladosporium cladosporoides contamination and you sample using a collection device that is inefficient at 2 µm sizes, you would probably get negative results because you would miss most – if not all – of the spores simply because the collection device is inefficient 2 µm. When contacting a laboratory or a vendor about sample collection methods and equipment, pay particular attention to particle size cut points and collection efficiencies.

b. Bulk samples – Bulk samples can be used, especially when trying to locate or confirm the presence of mold as a causative agent for medical diagnosis. The sample is analyzed by direct microscopic examination to determine if there is biological contamination. Bulk samples can also be cultured for identification. Examples of materials that might be collected include carpet, insulation, duct lining, wallpaper, wallboard (sheetrock), liquids from heating/ventilation/air conditioning (HVAC) systems, or dust.

(1) Solid bulk samples can be collected from visibly contaminated surfaces by scraping or cutting with clean tools (e.g., wall board). Place sample in a sterile container (plastic bag, glass or plastic vial, etc…) seal tightly and label for transport.

(2) Bulk water samples can be collected from condensate drain pans, cooling towers (i.e., for Legionella), or other water reservoirs suspected of being a contaminant source. Collect in a sterile container, seal tightly, and transport in a secondary container (such as a sealable plastic bag) to contain the sample in case of breakage or leaks.

(3) Bulk dust samples can be taken using a microvacuum. This is essentially a cassette attached to a pump that is used to vacuum carpets, furniture, or other substrates to collect the particulate matter. Though the sample can be randomly vacuumed into the cassette, using a specific grid collection area will allow quantitative results. This type of bulk sampling is often done on carpets.

c. Surface samples

(1) Surfaces can be sampled by swabbing or using clear cellophane tape (also called a "sticky tape" sample). The sample is analyzed by direct microscopic examination to determine if there is biological contamination. Sterile swab collections can also be cultured for identification. Surface sampling is limited to identifying settled fungi or spores and may not be related to air sample results. Sticky tape sample results provide spore identification to the genus level only.

(2) Settling plates/gravity plates are open nutrient agar Petri dishes. They are placed on a flat surface to collect anything that settles out of the air. Results are not

particularly meaningful, since what grows depends on random settling of airborne particulates onto a non-specific growth medium. Navy personnel will not typically use this method.

d) General sampling tips

(1) You have to decide whether you want viable or non-viable analysis or other specialized testing before you sample.

(2) Before collecting any samples, select the analytical laboratory you will use. Call the laboratory to ensure that you sample according to their requirements.

(3) In some cases, the laboratory may provide the sample collection equipment. For example, a laboratory might loan you an "Andersen" N6 and provide the correct agar for the targeted biological population.

(4) Sampling conditions should be reflective of "normal" building conditions. The ventilation system should be on the usual daily setting (i.e., temperature, damper opening(s), setbacks, auxiliary/booster fan operation, fresh air intake settings, etc...) and employees should work as they typically do. DO NOT intentionally alter the area to be sampled.

(5) Sample on different days and at different times of the day to get samples that represent conditions over time. Replicate samples are a good idea to increase confidence in your results. Remember that results tend to be less reliable or repeatable when sample times are very short!

(6) Aggressive sampling is not recommended for investigational studies. While aggressive techniques will disturb accessible biological contaminants, it complicates result interpretation because it is not representative of normal building conditions.

(7) Record ambient conditions during sample collection, such as temperature and relative humidity. Also, make notations of conditions inside that may affect results, such as obvious water damage or contamination in relation to the sample location; potential microbial reservoirs, like fish tanks, plants or trash; condition of HVAC system components; presence of pets; or open/ leaky doors and windows. Outside sample notes should include weather conditions (cloud cover, recent precipitation, wind, etc…) and locations of land features (ditches or standing water, landfills, playgrounds, construction areas, etc…).

(8) Chain of Custody (COC) - It is prudent to use a COC form with your samples. The COC is particularly important should you become involved in litigation, but should be used anyway to track the samples' journey from collection to analysis. If you do not have a COC form, most laboratories will supply you with one. You can view examples at the following sites*:

http://www.aerobiology.net/docs/2012%20COC%20-%20Editable.pdf
http://www.emlab.com/media/resources/submit.pdf
http://www.emsl.com/ChainOfCustodyForms.aspx
http://www.testamericainc.com/media/63924/TestAmerica_E-CoC.xls

***This is not an endorsement of particular products or vendors, and does not imply endorsement by the Department of Defense, the Navy, or the Navy and Marine Corps Public Health Center, nor does it mean that these resources' content have been validated.**

HAVING THE SAMPLES ANALYZED

Use only analytical laboratories that are proficient in the AIHA Environmental Microbiology Proficiency Analytical Testing (EMPAT) program and accredited by the AIHA Environmental Microbiology Laboratory Accreditation Program (EMLAP).

1. The EMPAT evaluates the laboratory's ability to identify cultured fungi and bacteria that might be found in mold contamination investigations correctly. The EMPAT certificate states whether the proficiency is for identification of bacteria, fungi, or both.

   Under the current program, laboratories must correctly identify the genus, and they receive bonus points for correctly speciating the organism.

   At this time, proficiency testing does not involve counting (of spores or colonies) or identifying organisms from mixed cultures.

   Beware of laboratories that advertise that they participate in the EMPAT rather than that they are proficient in the EMPAT.

   For the most current information, consult the AIHA web site at http://www.aihapat.org/ProficiencyTestingPrograms/empat/Pages/default.aspx.

2. The EMLAP assesses and rates various laboratory parameters, such as: personnel qualifications, EMPAT scores (performance), facilities, quality assurance programs, record-keeping, analytical methods, and operating procedures. EMLAP also includes biennial site visits to the laboratory.

   Laboratories can become accredited in one or more several fields of testing (FoT):

	FUNGAL By Laboratory Culture	**BACTERIAL By Laboratory Culture**	**FUNGAL By Direct Examination**
FoT	Air	Air	Air
	Bulk	Bulk	Bulk
	Surface	Surface	Surface

For the most current information, consult the AIHA web site at http://admin.aihaaccreditedlabs.org/programs/Pages/EMLAP.aspx.

A list of accredited laboratories is provided on the AIHA web site at http://admin.aihaaccreditedlabs.org/Pages/ListofAccreditedLabs.aspx. This list shows points of contact for the laboratories and the FoTs and methods in which the particular laboratories are accredited. Please consult directly with the laboratories for more specific information on analysis methods, and for information on additional analyses (Allergens, Endotoxins, Mycotoxins, MVOCs, etc…) and services available that are not listed on the accreditation.

3. Always check with the laboratory to confirm what services are available and what procedures should be followed. Define sample collection methods, procedures and equipment and how samples should be transported.

   Always check with the laboratory to confirm turnaround time and what is included in the cost. Some laboratories might offer sample collection equipment loan or rental programs if you use their analytical services. Some laboratories might include collection media (especially agar plates for viable sampling) in the analysis cost whereas others require you to purchase media separately.

   Furthermore, make sure that you know what the laboratory includes with their analysis. For example: If you submit a viable sample collected with an Andersen N6 impactor, will the lab identify all microorganisms or only the predominant 3 (or 5)? Will the report include genus and species identification or only the genus? Will the laboratory clearly report genus and spore count or spore concentration (e.g., Cladosporium 450 spores/m^3 or report results as Cladosporium-like (not definitive for Cladosporium but spores look similar) or Cladosporium 42 spores (you have to calculate concentration))? Also, some laboratory reports include interpretive guidance; some will not give any explanation, and still others will interpret your results for an additional fee.

INTERPRETING RESULTS

The presence of mold or other biological contaminants does not mean that occupants will have adverse health effects or that they will even be exposed. Like any other stressor, you must have a completed exposure pathway to the contaminant. The mold or mold fragments, spores, bacteria, metabolites or allergens must be produced, released, reach the occupants, then be inhaled, physically contacted, or ingested. Even after contact, human response will depend on individual susceptibility (e.g., genetic predispositions to allergens, age, health status), and type of exposure (allergen, toxin, infectious agent).

There are no standards for biological sample results. The AIHA, American Conference of Governmental Industrial Hygienists (ACGIH), Environmental Protection Agency (EPA), and numerous other resources agree that the best criteria for interpreting results is to compare inside samples with outside samples and/or contaminated areas with uncontaminated areas, along with consideration of both the kinds of biological contaminates present (such as molds genus/species)

and the numbers (quantitative assessments). Following are some interpretation criteria, specifically for molds.

1. **Interpretation Criteria for Molds**

   a. Compare Indoor and Outdoor Results - An effective interpretation is based on comparing inside and outside sample results. In general, inside counts should be around 30-80% of outside and have the same general distribution of genera.

      (1) Rank order the genera/species results. The relative order inside should be similar to outside. If the dominant types of mold in indoor samples are not the same as those in outdoor samples, it indicates an indoor mold source.

      (2) The concentration of each genus/species identified inside should be less than outside. Higher inside levels indicate there is fungal amplification indoors.

      (3) The presence or absence of a few genera in small numbers should not be considered abnormal.

      (4) Normal outside fungi typically include Cladosporium, Alternaria, Epicoccum, and Basidiomycetes, so it is common to see these identified in indoor samples.

      (5) The presence of certain fungi indoors should prompt immediate risk management decisions. Examples of fungi of concern include Aspergillus versicolor, Aspergillus flavus, Aspergillus fumigatus, Stachybotrys chartarum, Fusarium moniliforme, Histoplasma, and Cryptococcus.

      (6) Numerical guidelines can be useful as a secondary interpretive resource when evaluating viable fungi sample results (i.e., reported in colony forming units per cubic meter of air (CFU/m^3)). These guidelines are from the Health Canada: Indoor Air Quality in Office Buildings: A Technical Guide 1995. Fungi levels in excess of these numbers do not mean that the conditions are unsafe or hazardous. Do not use these guidelines for non-viable fungi sampling results.

         < 150 CFU/m^3 total fungi is acceptable if the reported genera are reflective of normal outdoor flora (e.g., Cladosporium and other leaf and tree fungi). Higher counts suggest dirty or low efficiency air filters or other problems.

         < 500 CFU/ m^3 total fungi is acceptable in summer if the reported genera are reflective of normal outdoor flora. Values higher than this may indicate failure of the filters or contamination in the building

         > 50 CFU/m^3 of a single species other than Cladosporium or Alternaria should prompt further investigation.

b. Consider Outside Air Entry.

(1) Filtered or conditioned air will affect the relative numbers of genera. In an office building with little fresh outside air or poor air exchange rates, 'normal' inside counts may be very low, i.e., 2-5% of outside. The rank order of genera should be similar.

(2) If sampling in a building or residence when doors and/or windows are open, expect 'normal' inside counts to be very similar to outside – as high as 95%. The rank order of genera should be similar.

c. Put Results in Context With Other Facts.

(3) On microscopic examination, morphologically similar spores cannot be differentiated. The most common example of this is with Aspergillus, Penicillium, Gliocladium, Trichoderma, and other small, round, colorless spores. Non-Viable sample results will report all such spores as Aspergillus/Penicillium group.

If non-viable sample results show high indoor counts of Aspergillus/Penicillium, you may want to collect viable samples for culture to separate the genera and determine which species of Aspergillus is present, since several produce mycotoxins and are infectious.

(4) Myxomycetes/Rust/Smut or Smuts/Periconia/Myxomycetes will also be reported together on non-viable sample reports. These are morphologically similar round, brown spores that are common outdoor plant molds.

(5) The presence of fungal fragments such as hyphae or conidiophores suggests colonization, growth, or accumulation of fungi in the sampling location.

(6) The presence of yeast suggests wet conditions.

(7) Be sure you know the ambient sampling conditions before using outside sample results:

(8) Outside samples collected during or soon after rain will usually have lower total spore counts but higher relative concentrations of ascospores and basidiospores.

(9) Expect higher concentrations of fungi in warmer weather, lower total counts in cooler weather.