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Mold Sampling and Diagnostic Service Indoor Air Quality Investigations

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Mold Biology

Molds are not plants or animals. They belong to the Kingdom of the Fungi. Other fungi include mushrooms, wood rot, and yeasts There is no such thing as "dry" rot. All wood decay fungi have high moisture requirements. Fungi digest organic material. These decay organisms recycle plant and animal matter. Many building materials are made from dead vegetables. To prevent fungal decay we must keep our buildings dry.

Molds digest their surrounding environment. They secrete digestive agents called "enzymes", which break down the environment into digestible pieces, which are reabsorbed. This is how a mold colony feeds and grows. The molds digestive enzymes require water to be chemically active. Mold can't feed on dry material. In order to get moldy, things have to be damp. The more the mold eats the more it grows. The colony can only feed on damp material, but can extend for short distances through dry material probing for more damp and digestible material.

Growth is slow at first, starting from a single spore or clump of spores. As the colony grows, it extends fine feeding tubes called hyphae into its food source. These tiny hairs can penetrate apparently solid material, such as cement and gypsum board core. The interconnected mass of hyphae is called the mycelium. When the colony is mature, it begins producing spores, which can then spread, by air movement or mechanical disturbance. Wet drywall may become covered with millions of spores per square inch in only a week or two if it stays wet and warm enough. Molds also require oxygen. Soaking wet material may not mold as rapidly as surfaces that are merely damp. Spores are often very tiny. A period in this text is about 100 microns in diameter. Common molds, such as *Penicillium* and *Aspergillus* species make spores that are less than 5 microns in diameter. Particles this small may take an hour to fall one yard. Moldy material will emit spores on an irregular basis. Thriving mold colonies also generate odors, but a space can be full of spores, yet have no discernable moldy smell. On the other hand, musty odors do not necessarily indicate heavy concentrations of spores. Once established, mold colonies can be very durable. When conditions for growth become less than optimal, the colony will produce a last crop of spores, then go dormant. This period of dormancy can last for years. When optimal moisture and temperature conditions return, the colony can come out of dormancy, the colony will come to life and begin to feed. The growth period will last as long as food, moisture and warmth are present. The bottom line: mold prevention is easier and cheaper than mold remediation.

Molds and Moisture

Visible mold is not always present in moldy environments. Mold thrives where it is damp, and exposed surfaces tend to dry more quickly. In water damaged indoor environments, mold is often found on the inside of a wall cavity, or on the underside of a carpet. Measuring the relative humidity in the center of a room may not tell us the potential for mold growth. Mold doesn't grow on thin air. What we are interested in is the moisture content of a digestible solid substance.

The moisture content of a solid material is called its water activity. Different materials have different moisture capacities. A piece of southern pine can only absorb about 30% of its weight in water before becoming soaked. This wood, with a 30% moisture content has a water activity of 1. As the water activity of organic material exceeds about 0.7, there is an increasing likelihood that mold will grow on it. Different fungi have different moisture requirements. Most yeasts grow best under liquid conditions. Rot fungi can only digest very damp wood. Molds that digest green leaves and grass, such as Alternaria and Cladosporium species require upwards of 90% water activity to thrive. Some *Penicillium* and *Aspergillus* species can colonize material with water activities as low as 70% to 80%.. As moisture content changes, a material may experience a succession of different molds growing on it. Timely response to a water disaster is critical if mold problems are to be avoided. Bulk water must be removed and the ambient air must be dehumidified. Then dry air must be delivered to all the damp cracks, crevices and cavities that are the prime spots for mold colonization. Once the drying process is begun, it is important that it be completed rapidly.

Moisture measuring technology is becoming increasingly accurate and affordable. When used in conjunction with appropriate dehumidification and air distribution equipment, almost any water disaster can be effectively remediated without any resulting mold problem. In determining the probability of a mold problem, we ask How wet for How Long? Totally soaked environments can be properly restored if they are dried soon enough. On the other hand, a little dampness can create big problems if it is permitted to persist.

Not all mold problems are the result of water disasters. Moisture problems can be the result of condensation, or capillary action. Cement readily transfers moisture from wet to dry. A cement dam may hold back the river, but the downstream side is wet to the touch. Capillary wicking can create damp, moldy conditions behind below grade walls, or under carpets. Such hidden reservoirs of microbial contamination can fill the indoor environment with unpleasant odors and clouds of potentially irritating spores.

Warm air holds more moisture than cold. Air at 75° F. and 25% relative humidity may seem dry. But when this air is cooled to about 40° F., it will be 100% humid. This is the driving force behind winter window condensation in heating climates. Such warm, relatively dry air can escape into a cold attic or wall assembly, where it can condense on cold building materials. If such areas stay wet enough for long enough, mold growth bed comes inevitable. On the other hand, hot humid air may have a dew point (condensing temperature) of over 70° F. Hot, humid air can work its way from outdoors through a wall assembly and condense on the back side of the cold vinyl wall covering in an air conditioned room. While vinyl may not be good mold food, wet sheet rock paper is

almost pure cellulose, one of the molds favorite meals.

Mold Sampling

Any sample is only an estimate of the true picture. In the case of microbiological sampling, the true picture can never really be known. The organisms we are measuring are usually invisible, and are often thriving in hidden places. When estimating mold problems, the most reliable approach is to follow the moisture. Without understanding the dynamics of the moisture problem, mold problems are difficult to either prevent or treat.

Sometimes the moisture that permitted the mold to become established is gone, but the mold is now established. Some detective work may then be required to "sniff it out". The first evidence of a mold problem in a home or building may be occupant health problems. Sampling may be recommended to estimate whether mold levels might be a causative factor.

The sampling method selected must answer the questions that need to be asked. Is the air quality a health threat? What is that black stuff on the window sill? What's that smell? There are basically three methods for evaluating mold. We can sample the air, we can test surfaces, or we can collect and assay bulk materials.

One common air sampling method transfers viable mold spores in the air onto microbiological media. Simply exposing a petri dish to the air in a room is not an accurate way to evaluate mold levels. Many common spores are too small to fall rapidly. The "settle plate" is prone to underestimating mold levels, and is never recommended. A good air sampler will force the spores to impact the collection surface. The sampler must also have a known flow rate, so that counts of mold recovered can be used to estimate the mold level in the area being measured.

A popular air sampler is the Andersen N-6. A petri dish loaded with a mold growth medium is placed in the sampler, which is then connected to a vacuum source. The sampler is designed to collect air at a flow rate of one cubic foot per minute. Petri dishes are then placed in an incubator. After an appropriate period of time, spores will grow into colonies, which can be counted and identified. Example: One colony is found to grow on a petri dish exposed to a one minute sample time in an Andersen N-6. A one minute sample collects one cubic foot of air. There are about 35 cubic feet per cubic meter time. The mold level would be reported as 35 colony forming units per cubic meter (35 CFU/m3).

While the Andersen has good collection efficiency, it can only measure those spores capable of growing on the petri dish. An appropriate medium must be selected, or spores may not grow well. In addition, an airborne spore may have lost its capacity to form a colony. While still capable of affecting occupant health, this spore would not be counted by a viable sampler, such as the Andersen N-6. For this reason, many investigators choose to collect non-viable samples as well. This can be done by using an instrument which draws a measured volume of air onto a sticky surface, leaving a trace of defined size. This surface can then be stained and examined microscopically, in order to count and identify collected spores. Results are reported in spores per cubic meter. Surfaces can also be evaluated for level of microbial growth. A sterile cotton swab, moistened with sterile water, can be wiped against a surface, or inserted into or

under something. The swab can then be streaked onto a petri dish, then incubated and evaluated like the Andersen sample. Heavy contamination is readily apparent after a few days to a week of incubation. This method also assumes viable mold, that is capable of forming colonies.

Visible mold can be easily identified using the tape tease method. A short segment of clear Scotch tape is placed sticky side out against the mold, then pulled away and placed on a stained microscope slide for direct examination. This allows the investigator to determine whether the suspect material is mold, and if it is, what kind. Sometimes, bulk material is collected for analysis. Example: A piece of insulation is collected from a water damaged wall cavity. The sample is bagged, labeled and taken to a lab. There, it is weighed and diluted and shaken in a known volume of sterile water. A small, measured portion of the water is then spread onto a petri dish. After incubation mold colonies are counted and identified. Results are reported in cfu/gram. This method also assumes viable spores.

The issue of viability is important in evaluating mold clean up jobs. Moldy surfaces are often treated with biocides, which may render spores non-viable. If carelessly handled, such treated surfaces can release clouds of spores into the surrounding air. While potentially a health hazard, these treated spores may not be measured by viable sampling methods.

Mold and Health

The earth is a moldy planet, and has been for over a billion years. Where there is life there must also be decay. The entire biosphere, from deep in the soil to high in the atmosphere is loaded with either fungal organisms or their spores. Humans have always been exposed to mold. If inhaled spores were not destroyed, some species would quickly colonize and digest the body. Spores are readily cleared by a variety of immune system responses.

The human immune system is vast, complicated and poorly understood. Taken cell at a time, all the various immune system components make the heaviest organ in the body. The immune systems main job is to preserve the integrity of the body. Foreign living tissue is not tolerated. On of the most important immune defenders is the antibody. Antibodies are complex folded proteins that serve rather like keyholes. They are trained to recognize and ignore our own tissues. The antibodies circulate throughout our tissues, constantly scanning for foreign proteins, which may be considered keys. When a particular antibody keyhole locks onto a foreign key, a series of events occur which end in the intruder being destroyed and eliminated from the body.

Allergy is a specific immune system disorder. People who have allergies make antibodies that overreact when they bind to foreign proteins. When they bind they trigger release of a number of toxic compounds, including histamines. A trivial exposure to a bit of cat dander, ragweed pollen or a mold spore triggers a cascade of toxic releases that make a routine bit of immune janitorial work into a war zone. It is as though the National Guard and the police TAC squad were called in, weapons blazing, to arrest a jaywalker. From 10% to 20% of the population is estimated to be allergic to some degree. Those who are allergic to mold can be quite miserable when exposed to levels of airborne

Those who are allergic to mold can be quite miserable when exposed to levels of airborne spores that are harmless to others. Symptoms may include headaches, sinus irritation,

sore throats, eye irritation, coughing, sneezing, skin irritation and nausea. While the outdoor air is always \moldy during warm weather, it seems that chronic exposure to the sorts of molds that tend to colonize building materials can make moldy indoor air particularly irritating. Allergy suffers who are miserable in a moldy home or building, may experience relief from symptoms outdoors, where spore counts may be as high or higher, but consist of different species. Prolonged exposure to moldy indoor environments has been shown to make some people very ill.

Molds have complex biochemistry. Some species manufacture toxic compounds called mycotoxins. These mycotoxins have a wide range of properties. The antibiotic Penicillin is actually a mycotoxin, produced by *Penicillium* species. The molds secrete this anti bacterial agent to kill off competitors for food; chemical warfare on microscopic scale.

Most mycotoxins have been discovered by veterinary researches feeding moldy food to animals. Eating moldy building materials is not recommended. No one knows what sort of harm is experienced by inhaling potentially toxic spores. There is no defined dose that presents a clear danger. This is an area of active research. While the risks are not well understood, it is generally accepted that people should avoid living in moldy environments. Cleaning professionals should take care to protect themselves against the clouds of invisible spores generated during the demolition of moldy building materials.

Remediating Moldy Environments

It is easier and cheaper to prevent mold than to remove it. In addressing an existing mold problem, the first task is to identify and, if necessary, eliminate the moisture problem that caused it. The extent of the problem must then be determined. Decisions must be made about what to clean and what to discard. If the problem is confined to one part of a structure, the abatement area must be contained, to avoid contaminating clean areas.

Deciding what to throw away is a challenging problem. Drywall, carpet, upholstery and other porous material are difficult to save once penetrated by mold. Wood, cement and other hard surfaces can usually be cleaned and treated with antimicrobials.

Perhaps the most challenging surface is interior lined fiberglass duct work. Ideally, all moldy lined duct work should be discarded, but cost considerations dictate that it be cleaned and treated in place.

There is currently debate over the fate of porous contents from moldy environments. Clothing, furniture and carpeting that have never been wetted, or supported actual mold growth can still become covered with mold spores if they have been in moldy environments. Some "experts" suggest that all such contents must be discarded. There is much evidence to suggest that such items can be cleaned of all deposited spores and returned to safe use.

There is always some sort of compromise between what is best practice and what is affordable. The cleaner should be aware of the options, and make their customers aware as well. As with most things, one gets what one pays for, but the best doesn't necessarily cost that much more. On the other hand, paying a lot doesn't guarantee a

good job.

Gutting and cleaning is messy work. Countless millions of spores can be released into the air during the cleaning process. The most important indicator of good clean up is a spotless job site. Debris and dust in a moldy environment is loaded with spores. Damp cleaning, HEPA vacuuming, and exhaust ventilation all contribute to a cleaner job. Portable HEPA air scrubbers can also help. Exhaust ventilation should be used to create "clean-to-dirty" air flow while moldy material is being either removed or surface cleaned. In addition to a visual inspection, the customer may require some sort of clearance testing at the end of the clean up. Air and surface samples should ideally demonstrate that there is no mold in the cleaned area with an indoor source. The problem is that the colonies recovered in samples come with no pedigrees. Indoor and outdoor mold tends to have a different species composition, but that ain't necessarily so. The contractor may have been hired to clean only a certain portion of a home or building. It is perfectly possible that there are other mold/moisture problems in the building that can effect air quality in the work area. Finally, there are no firm criteria for what is good or acceptable in terms of indoor mold levels. The best protection for vendor and customer is to have all terms and expectations carefully defined, to avoid surprises or disappointments.