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A Comparison of Particle Counts Obtained with a Direct Reading Particle Counter to Mold Spore Counts Obtained with an Integrated Spore Trap Method

By:

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Abstract

This study was conducted to determine if a correlation could be made between particle counts collected with the Lighthouse Handheld 3016 IAQ direct reading particle counter and spore counts collected with an Air-O-Cell spore trap cassette.

Thirty samples were collected in a cabin in Southwestern Montana in the month of December 2004. The cabin was heavily contaminated with visible mold growth. The direct reading particle counter selected for this study was the Lighthouse Handheld 3016 IAQ particle counter. The particle counter sampled at six different size channels; 0.3 μm, 0.1 μm, 1 μm, 2.5 μm, 5 μm, and 10 μm. The Air-O-Cell cassette was used to sample the mold spore counts in the area side by side with the direct reading particle counter.

Cumulative concentrations at the 2.5 μm cut point were used from the direct reading instrument and compared with Air-O-Cell spore concentrations. A moderate positive correlation (.536) was found between the Air-O-Cell spore concentrations and the handheld 2.5 μm particle concentrations (p=.003) A weak R-Squared value (.287) was also found.

Cumulative counts at the 2.5 μm cut point were used from the direct reading instrument and compared with the Air-O-Cell spore counts. A strong positive correlation (.787) was found between the Air-O-Cell spore counts and the handheld 2.5 μm particle counts (p=.000). A strong r-squared value (.619) was also found.

The correlations identified between mold spore counts and 2.5 μm particle counts warrant further research in this area. This research may prove valuable in the ability of direct reading instrument to predict mold spore counts.

Acknowledgments

I would like to thank all my committee members for their continuing support and guidance on my thesis: Professor Julie Hart, Dr. Terry Spear, Professor Amy Kuenzi and Marilyn Cameron, without your support this thesis would not have been possible. A special thanks to Julie Hart for giving your time and expertise to guide me through this project. I would like to thank Judy Murphy for her help and expertise throughout my research. I would also like to thank my family, friends, and most of all my husband for all their support and encouragement throughout my academic career.

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1.0 INTRODUCTION

1.1.1 Mold History

Molds are a member of the fungi kingdom, which also includes mushrooms, mildews, and yeast. Mold is found everywhere in our environment, and in nature, it performs the vital function of breaking down organic matter. [1] [2] Without mold, the environment would be littered with leaves, grass, and other organic matter. Molds have also served other important purposes for mankind.

Many fungi have been used for centuries in a variety of useful applications. Yeast is an essential ingredient in bread, beer, and wine. Mushrooms are used in many dishes for added flavor. The first antibiotic and bleu cheese were derived from mold growth on food. [2] While many fungi are beneficial in everyday life, deleterious effects have been documented throughout history.

In the Bible, Leviticus chapters 13 and 14, a house that had mildew was first cleaned and then if the mildew was still present the "unclean" stones were removed and replaced with clean stone. If the mildew returned, the house would be torn down and all material was removed from the town. [3] [4] In Salem 1692, several individuals were put to death and many more were tried for witchcraft. The bewitchment of the first accused is now believed to be linked with digesting contaminated rye kernels that had produced mycotoxins. Ergotism is caused from eating rye that has been infected with the fungus Claviceps. The Irish

Potato Famine of 1845 was the result of a fungus called *Phyophthora infestans* that turned the potato crops into black rot. The "curse of the mummy" can also be attributed to several species of fungus. Casimir's tomb was opened in Poland in 1973. Twelve researchers were present when the tomb was opened and only two survived. When the tomb was examined, Aspergillus flavus, Penicillim rubrum, and Penicillium rugulosum were present. These fungi are known to produce aflatoxins B1 and B2 and are the primary suspect in these deaths along with several other "mummy curse" casualties. [3] [4] The following sections will give a brief introduction on mold biology and discuss mold species.

1.1.2 Mold Biology

Molds are microscopic eukaryotic organisms that are not capable of making their own food. They require moisture, oxygen, nutrients, and ideal temperatures to thrive. [2] Moisture is the most important requirement for mold growth; without moisture mold can not thrive. Water activity (a_w) is the amount of free water available on a substrate to support mold growth. Water activity is defined as the ratio of the amount of water in a material at a particular temperature and pressure to the maximum amount of water that can be held by the material at the same temperature and pressure. Fungi species have different water activity (a_w) requirements. A water activity level of 0.66 and up can sustain mold growth. [5]

Mold spores float through the air and eventually land on a surface. If the surface is moist, the spore will begin to produce enzymes that digest the substrate into smaller molecules that can be absorbed by the spore as nutrients.

Once the spore begins to germinate and grow, it will begin to reproduce. Fungi reproduce sexually as a spore and asexual as conidia. Both are commonly referred to as "spores". [4] Mold spores reproduce rapidly and easily adapt to changing environmental conditions. When environmental conditions become too harsh, many of them can go into "hibernation". When optimal conditions return, the mold will begin to thrive.[2] Many materials found inside structures provide nourishment and support mold growth when optimal conditions are present. Over 1,000 different molds can be found growing inside structures.

1.1.3 Mold Species

There are many types of mold that can be found indoors, since mold is found in almost every environment. Mold is brought into a structure from natural air currents, open windows and doors, ventilation systems, and movement of occupants through the structure. Several molds commonly found indoors include Cladosporium, Aspergillus, Alternaris, and Penicillium. [6] All of these species have several genera that can be found in most structures.

1.1.3.1 Cladosporium sp.

Cladosporium sp. is the mold species commonly found outdoors in soil with twenty eight to forty different species in existence world wide (see Figure 1). Cladosporium is considered a dry spore and is easily dispersed through the air with a size range from 3-30 microns (μm). [7] [8] The water activity level for Cladosporium is 0.85-0.88 and is associated with refrigerated foods which can grow at 0°C. Cladosporium causes Type I allergies (hay fever, asthma) and Type III hypersensitivity pneumonitis. *Cladosporium herbarum* enzymes transform steroid intermediates of progesterone to be used in the production of oral contraceptives. [7] [8]

Figure 1: Cladosporium sp.
Source: Environmental Microbiology Laboratory, Inc

1.1.3.2 Aspergillus sp.

Approximately two hundred species of Aspergillus exist and can be found in soil, decaying plant debris, and grain (see Figure 2). Water activity ranges from 0.71 -0.94 with spore sizes from 2-12 μm depending on the species. Spores are dry and are easily dispersed in the environment. [8] *Aspergillus sp.* causes Type I allergies (hay fever, asthma) and Type III hypersensitivity pneumonitis. *Aspergillus fumigatus*, can cause allergic bronchopulmonary aspergillosis (ABPA) and allergic fungal sinusitis. [7] Many species of Aspergillus grow well at body temperature causing invasive function. Three types of diseases have been recognized from exposure to Aspergillus: infection in living tissue, allergic reaction, and toxicosis due to ingestion. *Aspergillus flavus* grows on corn and peanuts producing aflatoxin B1 & B2. *Aspergillus niger* is used in the bread and beer industries and cortisone production. [7] [8]

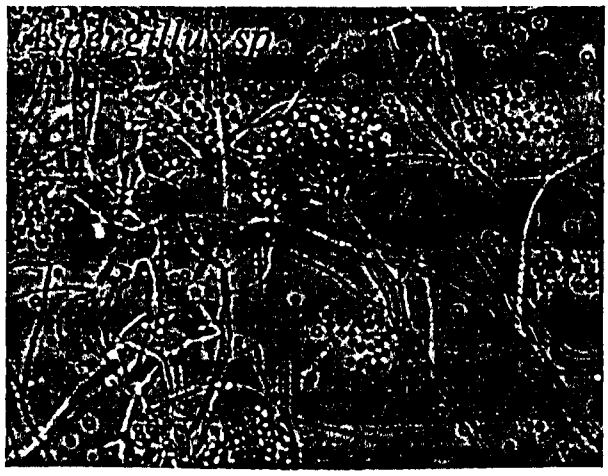

Figure 2: Aspergillus sp.
Source: Environmental Microbiology Laboratory, Inc

1.1.3.3 Alternaria sp.

Alternaria sp. is reported to cause Type I allergies, Type III hypersensitivity pneumonitis, nasal and subcutaneous lesions, and nail infection (see Figure 3). [7] Species range in size from large spores 20-200 μm depositing in the nose, mouth, and upper respiratory tract to 7-18 μm which can deposit deeper in the lungs. Alternaria grows well on dead organic debris, textiles, and food stuffs with a water activity ranging from 0.85 -0.89. Industry uses some *Alternaria sp.* for bio-control of weeds and other plants. [7] [8]

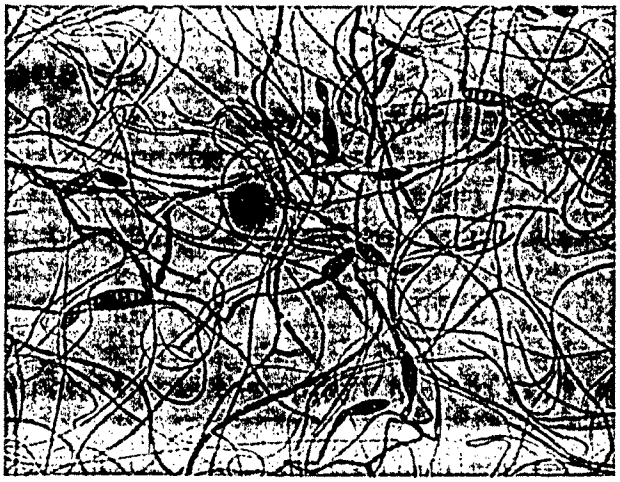

Figure 3: Alternaria sp.
Source: Environmental Microbiology Laboratory, Inc

1.1.3.4 Penicillium sp.

In 1928, Sir Alexander Fleming observed mold growing on Petri dishes in his lab. He observed the mold was killing bacteria and prohibited further bacteria growth. The species was later identified as Penicillium in which the antibiotic

penicillin is isolated (see Figure 4). [4] [7] There are approximately two hundred species of Penicillium found in both indoor and outdoor environments. Species can grow on many different substrates and food products with a water activity of 0.78-0.86. Some species cause Type I allergies and Type III hypersensitivity pneumonitis. Industrial uses include salami sausages starter culture and roquefort and camembert cheese production. [7] [8]

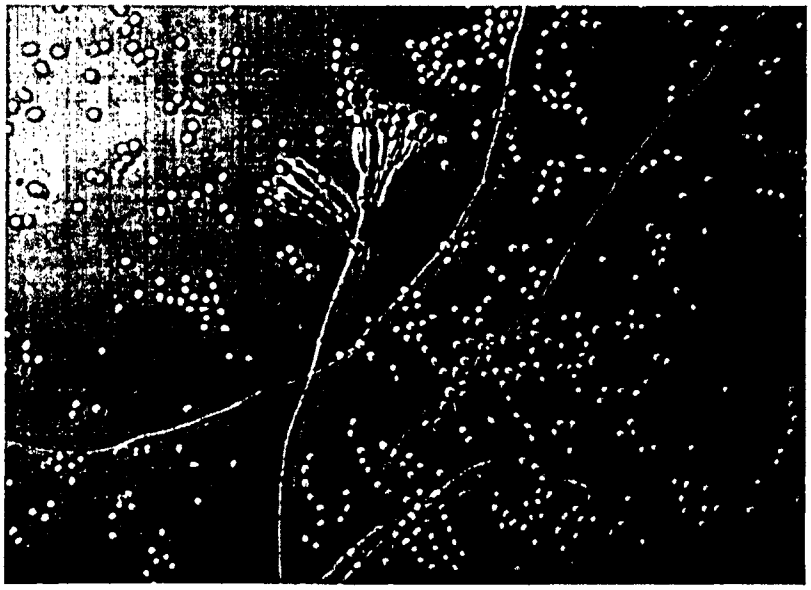

Figure 4: Penicillium sp.
Source: Environmental Microbiology Laboratory, Inc

1.1.3.5 Toxigenic Fungi

Some species of mold are capable of producing compounds called mycotoxins. Mycotoxins are classified as secondary metabolites because they are not necessary for spore growth. Mycotoxins are produced as a defense mechanism against competing organisms. [9] Mycotoxins are produced in various

parts of a spore, and killing the spore does not eliminate their toxicity. [4] These mycotoxins have several toxic effects on both humans and other organisms. Mycotoxicosis is the term used to describe these toxic effects. Symptoms reported from exposure include recurring flu-like symptoms, sore throat, headache, fatigue, diarrhea, and altered immune function. [10] [11] The severity of toxic effects on an individual depends on several contributing factors. Some of the most important factors include the toxicity of the mycotoxin, the duration and concentration of exposure, and the susceptibility of the individual. The toxicity of the mycotoxin is dependent on the species of mold, environmental conditions, and quantity of myctoxins being produced. [9] Several species of mold produce mycotoxins and many can produce more than one type of mycotoxin.

Several members of the Aspergillus genus produce the mycotoxin aflatoxin. Aflatoxin B1 is a natural carcinogen that can be biotransformed in the body after ingestion of contaminated peanuts or corn. The biotransformation primarily occurs in the liver and is linked to hepatocellular carcinoma. [9] [11] [12] Most published studies on aflatoxin exposure have been associated with the ingestion exposure. In 1978, Mcmillan et al. measured aflatoxin B1 in concentrations up to 4700 ng/g in grain dust. In a recent study conducted by Selim et al. aflatoxin B1 was measured in concentrations up to 5100 ng/g in settled dust of farms. [13]

Ingesting rye products contaminated with ergot alkaloids produced by *Clabiceps purpurea* causes ergotism; a condition characterized by diarrhea,

nausea, vomiting, and abnormal cardiac rythms. In severe cases it can cause gangrene of the limbs, convulsions, hallucinations, and death. [9]

The fungus *Penicllium chrysogenum* produces the mycotoxin Penicillin used as an antibiotic. The antibiotic kills bacteria and inhibits bacterial growth in the human body. [7] [10]

Stachybotrys chartarum is a sticky greenish-black mold that is not easily aerosolized (see Figure 5). There are approximately fifteen species of stachybotrys with a water activity level of 0.94. Most medical research has been conducted in the agricultural industry from livestock ingesting hay and grain contaminated with Stachybotrys. [7] [8] In the 1940's a dermatologic and respiratory syndrome developed in fodder-handlers and those in close contact with musty straw. This suggested a route of entry through direct contact or inhalation rather than ingestion. [14] Hodgson et al. reported mucosal irritation, fatigue, headache, and chest tightness in patients who had occupied a building contaminated with Stachybotrys. The symptoms occurred within weeks after moving into the building. [14] Type I allergies have been reported from human exposure. [7] Many species of Stachybotrys produce very powerful mycotoxins earning the species the title "toxic black mold".

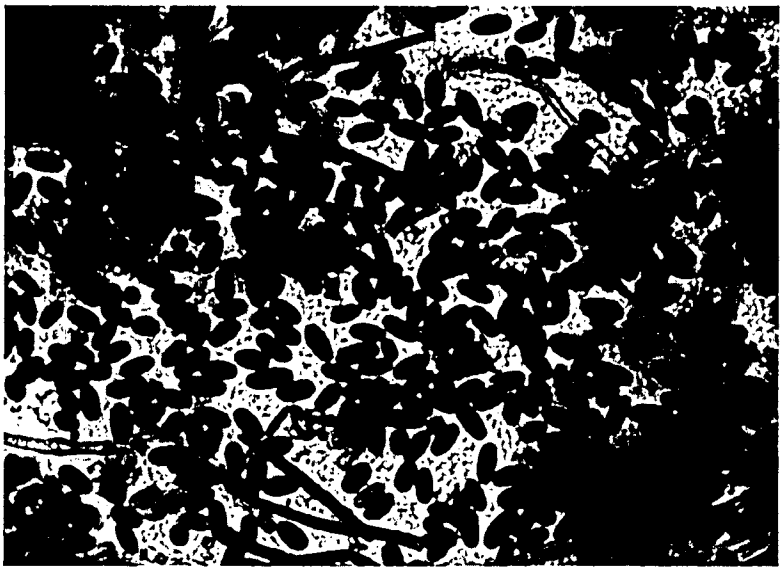

Figure 5: Stachybotrys sp.
Source: Environmental Microbiology Laboratory, Inc

1.2 Health Effects

Exposure to mold can cause many health effects. Molds produce allergens, irritants, and toxicants that can be harmful to humans and even pets. Some health effects that have been linked to mold exposure include: building related symptoms, hypersensitivity diseases, & respiratory system irritation.

1.2.1 Building Related Symptoms

Building Related Symptom is a broad term used to describe the symptoms associated with occupants residing in contaminated buildings. Generally, symptoms affect the individual while inside the contaminated structure and subside once the individual leaves the structure. The severity of the symptoms vary depending on individual susceptibility and the concentration and type of

molds present. [9] [15]

A study by Platt et al, found that occupants of moldy buildings had an increase in health related complaints. In a separate study, 6,000 children in six states also suffered health issues when occupying contaminated structures. The children complained of headache, eye irritation, nasal and sinus congestion, cough, and cold and flu symptoms, and gastrointestinal disorders. [14]

Allergy symptoms are common in susceptible individuals. Molds that produce mycotoxins can cause symptoms in those individuals that may not have an allergy to mold. Symptoms individuals may experience include headache, fatigue, rashes, and eye, throat, and nose irritation.[9] [15]

Two teachers working in a rural school that was contaminated with mold complained of symptoms when inside the structure. The first teacher developed sore throat, hoarseness, cough, wheezing, and shortness of breath. The symptoms continued over a three year period during the school year with symptoms subsiding in the summer months. Another teacher in the same school reported chronic cough, bronchitis, and sinusitis progressively worsening over a six year period. The teacher reported her symptoms improved after leaving the school. Sampling was conducted and mold levels were found to be much greater than outdoor concentrations. The species present in the highest concentration included: Paecilomyces, Penicillium, Aspergillus, and Stachybotrus. [16]

An office worker had complaints of sneezing, coughing, dizziness, fatigue, headaches, upper respiratory irritation, and rashes. Her office was located in a

mold contaminated area that was under remediation. She was moved into a "clean" area and symptoms improved for a short time. Paper work and other items from the contaminated area were brought to the new office and symptoms began to occur. She later was moved into a different building and her symptoms slowly improved. [16]

Contamination of ventilation systems can cause several individuals to fall ill throughout the structure, making it hard to determine the source of the contaminant. In many cases, building related symptoms are dismissed as other illnesses until the symptoms continue for some time. [9] [15]

1.2.2 Hypersensitivity Diseases

"Hypersensitivity refers to pathologic processes that result from immunologically specific interactions between antigens and humoral antibodies or sensitized lympocytes." [17] These diseases occur when an individual is exposed to an antigen. The individual must be sensitive to the antigen in order for an adverse reaction to take place. The antigen stimulates the production of specific Immunoglobulin E (IgE) antibodies and causes the individual to experience an allergic reaction. Some individuals can have serious allergic reactions when exposed to the antigen. Three hypersensitivity diseases associated with mold exposure include allergies, asthma, and hypersensitivity pneumonitis. [15] [16]

1.2.2.1 Allergies

Many species of mold are classified as allergens. Inhaling or touching mold spores of these species may cause allergic reactions. Sensitive individuals make antibodies that react when they bond to foreign proteins, triggering the release of histamine. Allergic reactions can occur immediately after contact or several hours after exposure. The reaction can range from mild to severe. The severity of the reaction depends on individual susceptibility. The symptoms of mold allergy are similar to hay fever. Symptoms include:

- Respiratory irritation
- Asthma
- Nose and throat irritation
- Nasal congestion
- Itchy, watery, or burning eyes
- Skin rash
- Hives
- Headache
- Fatigue

Mold allergies can be difficult to diagnose even with allergy skin testing. The reaction is specific to the mold species, so the susceptible individual must be tested with the mold species to be diagnosed. Allergy related asthma can also occur in susceptible individuals. [15] [18] [19]

1.2.2.2 Asthma

Asthma is an inflammatory respiratory illness that causes inflammation and narrowing of the bronchial tubes. Extrinsic asthma (allergic asthma) is triggered when a susceptible individual is exposed to an allergen such as mold spores. Symptoms include coughing, wheezing, shortness of breath, and chest tightness. Asthma symptoms have been reported in individuals who do not have a history of extrinsic asthma. [19]

A study conducted on 1,002 infants concluded that infants who were exposed to high levels of Penicillium were at significant risk for developing wheeze and persistent cough. [20]

A one-month old infant developed upper respiratory symptoms and non-productive cough. The child was treated in the hospital, responded well and was sent home. Once at home the child again became ill. After several trips to the emergency room and stays in the hospital, concerns developed about the home environment. Fungal growth was present in several rooms in the home. The child was discharged from the hospital and did not return to the contaminated home, making a full recovery. [16]

Mold spores can also trigger respiratory illness such as infectious respiratory diseases and Hypersensitivity pneumonitis.

1.2.2.3 Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis is caused from exposure to organic dust. Farmer's Lung is an example of hypersensitivity pneumonitis. Farmer's Lung is an allergic disease caused from inhaling moldy dust. The disease is noninfectious and is considered a disease because of the way the body reacts to the invading mold spores. The moldy dust comes from straw, grain, hay, and bird droppings. Mold spores attach themselves to dust particles that are stirred up into the air during work. Due to the small size of the mold spores, they are inhaled into the deep lung. Once imbedded in the lung tissue they begin to produce toxicants. These toxicants can interfere with immune system responses and may affect the macrophage defense mechanisms. The symptoms are similar to the common cold and include fatigue, fever, shortness of breath, and chest tightness. [15] [16] [21]

Due to the health concerns of mold exposure, identifying problem areas and removing contamination are important practices to avoid many health problems associated with mold exposure.

1.3 Sampling and Inspection Tools

The presence of mold may be evident in some cases and almost invisible in others. This is why a mold assessment must be conducted. An assessment involves a visual inspection and can include one or more of the following: bulk or surface sampling, moisture sampling, or air sampling.

Indoor air quality has posed problems for many home owners and businesses. Locating the cause of the problem is not always easy. Mold is only one of many possible indoor contaminants that may be present inside a structure. The protocol for mold sampling has changed continually over the years. The view on when to sample for mold is just one of the changes made in the last couple of years. Currently, if visible mold is present in the structure, mold sampling is not generally necessary unless the occupants are suffering adverse health effects and the species of mold must be known. The previous protocol was to sample even if visible mold was present in order to determine species and concentration of contamination. The species does not determine the type of remediation; all remediation is performed in the same manner regardless of mold species present. [22] [23]

1.3.1 Visual Inspection

The first step in mold assessment is the visual inspection. The inspection begins on the outside of the structure. The roof, gutters, and siding are inspected for possible water leaks. The drainage around the structure is inspected for sources of water or moisture entering the basement. Water entry from these areas provides excellent conditions for mold to spread and grow out of control before any signs are detected in the interior of the structure.

The interior of the structure is inspected for visual signs of mildew and water staining. Plumbing is inspected for leaks and for water damage that may

have occurred from past plumbing problems. In residential dwellings, carpeting in the bathroom and laundry areas are examined for water stains and excess moisture. Bathroom and dryer vents are inspected to ensure they are properly vented to the exterior. If used in the structure, humidifiers and dishwashers can increase surface moisture and should be noted as possible moisture sources. The relative humidity inside the structure is taken to determine if humidity levels are elevated. Musty or moldy smells can indicate problems areas. Furniture may have to be moved to determine if mold growth is visible behind objects in a smelly room. Windows should be inspected for leaks or openings that may be contributing to increased moisture levels. Once the visual inspection is complete, further analysis and sampling may need to be conducted depending on the scope of the problem. [24] [25]

1.3.2 Bulk and Surface Sampling

Bulk samples are taken from materials containing visible mold. A small portion of the material is removed and placed in a clean, sealed bag.

Sterile clear tape is used to take surface samples from suspect material. Clear tape must be used so spores are properly identified and seen under the microscope. A few inches of clear tape are applied to the surface using light pressure. The sticky side of the tape must not be contaminated by touch. The tape is slowly removed from the surface and placed in a clean sealed bag. Each sample is placed in a separate bag.

Wipe samples are another means of obtaining surface samples. Wipe sample kits contain a swab, surface grid, and swab tube. The grid is placed on the surface and the swab is rolled over the entire surface (see Figure 6). The swab is then placed in the tube and sealed. [26] [27]

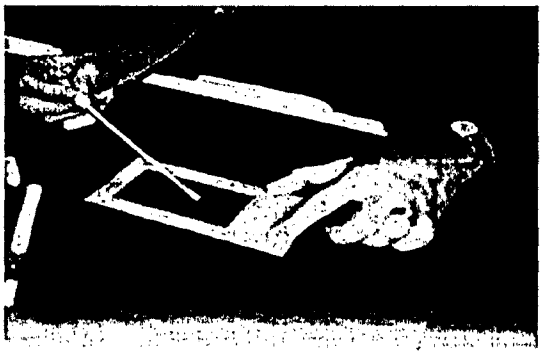

Figure 6: Surface or Wipe sample
Source SKC Inc. 2004

1.3.3 Moisture Sampling

A moisture meter can be used to detect hidden moisture behind surfaces. Moisture meters can detect the percent of moisture in walls, concrete, gypsum, wood, sheetrock, brick, and many other surfaces. There are many types of moisture meters available with varying features. The moisture meter pictured below is versatile and easy to use (see Figure 7). The Protimeter Surveymaster SM moisture meter features the two-pin inspection method along with a radio frequency technique. This model provides a variety of methods to sample for moisture.

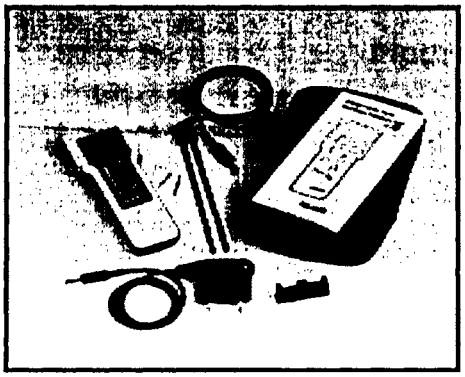

Figure 7: Protimeter Surveymaster SM
Source: Protimeter Plc 2004

When the two-pin mode is used, the prongs are pushed into the surface of the material to obtain the readings.

The Protimeter 'Heavy Duty Moisture-Probe and Lead' can be used to take measurements in hard to reach places. The Protimeter 'Deep Wall Probes' can be inserted deep into a surface to get moisture readings at different thickness levels to determine saturation (see Figure 8).

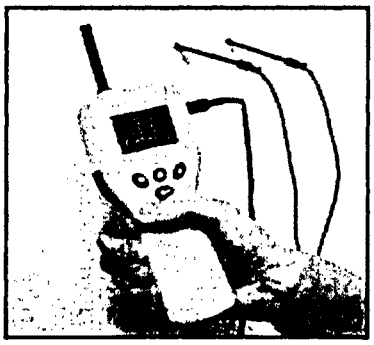

Figure 8: Probes in Wall
Source: Protimeter Plc 2004

This meter will also take moisture readings using non-invasive radio frequency emissions (see Figure 9). Moisture meters are used to find the source of moisture in an area. They do not detect the presence of mold. Air sampling is required to determine if mold is present in the air. [28]

Figure 9: RFE Method
Source: Protimeter Plc 2004

1.3.4 Cultural Sampling

When different species need to be identified, cultural sampling is used to obtain this information. Spores are collected on microbiological media plates and cultured to determine what types of mold are present in the air. [26] Standard agar media used for culturing spores includes Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Dichloran Glycerol 18 Agar (DG-18). [8] [29] This can be done with several types of bioaerosol impactors. The Anderson N-6 Bioaerosol Sampler, BioStage-1 Bioaerosol Impactor®, and the BioCassette are three types of samplers used to collect viable spores.

National Institute for Occupational Safety and Health (NIOSH) Method 0800 should be followed when sampling for viable microorganisms. [7] [8] A high flow sampling pump is used to pull air at 15 liters per minute (L/min) through multiple jets over the top of a petri dish containing agar media collecting the spores. The petri dishes are then collected and sent to a qualified laboratory. The petri dishes are placed in an incubator for a period of time and are grown so the entire colony can be examined for identification. The results are reported in

concentrations of colony forming units per cubic meter of air (CFU/m^3). [30]

When sampling with agar plates, an outdoor sample is taken for comparison. This practice is valuable to determine the species of mold present in a contaminated area compared to species present in the outside environment.

This method has some disadvantages that must be noted. Some types of mold spores grow very slow or not at all on some media. Stachybotrys is one type of mold that does not grow well on potato dextrose agar. Molds that grow well on standard media can over grow other species and underestimate the actual spore counts in an area. [6] This method does not measure non-viable or dead spores that can be just as dangerous as viable spores. In addition, this method may underestimate the amount of mold present in an area. [5] [26]

More than one sampling method may be used to evaluate a mold problem. The collection of viable spores, non-viable spores, pollen, skin, and other particles may be necessary to get a more accurate account of a contaminated environment using a spore trap method.

1.3.5 Spore Trap Sampling

Spore trap samplers collect both viable and non-viable spores on the collection media. Spore trap methods operate on the principle of inertial impaction. The particle-laden air enters the sampler through the inlet and flows toward the impaction slide. The air is reflected around the collection slide, and particles with sufficient inertia deviate from the air stream and impact on the

glass slide. Smaller particles continue in the air stream and exit the sample. [31] Several types of media can be used to collect non-viable data. The Allergenco, Burkard, and Zefron Air-O-Cell samplers are a few samplers available for this type of analysis.

The Allergenco and Burkard samplers operate on the same principle using glass-slides coated with a sticky material. Both samplers require the user to coat the plates prior to sampling. The sampling plates are placed inside the samplers, and air is pulled through the sampler making a 90 degree turn at the surface, collecting particles on the glass-slide. Once the sample duration is complete, the plates are removed from the sampler and sent to a laboratory for analysis. [7] [8] [29] The sampling devices are used over again with new glass-slides.

The Zefon Air-O-Cell cassette contains a sticky sampling medium that adheres particles to the slide (see Figure 10). Air flows into the cassette and makes a 90 degree turn at the surface of the sticky slide. The slide can then be analyzed for viable and non-viable spores, pollen, insect fragments, skin cells, and many other particles. The Air-O-Cell cassette is a single use sampling device. The cassette comes preassembled, and the entire cassette is sent into the lab. [31] Air-O-Cell cassettes can also be used with a WallChek™ Microbial Sampler. This instrument provides a non-destructive way to sample in wall cavities and under carpets. If moisture readings are elevated behind a surface, the Wallchek™ can detect if mold is present.

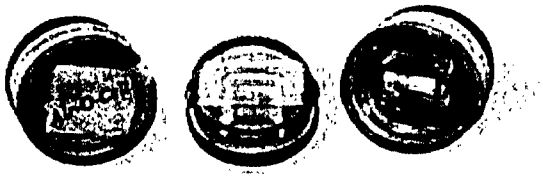

Figure 10: Air-O-Cell Cassette
Source: SKC Inc. 2005

The cut point for the Burkard and Air-O-Cell samplers are very similar at 2.52 μm and 2.3 μm respectively when calibrated at 15 L/min. Both samplers have low collection efficiency below the cut-off diameter. Collection efficiency above the cut off is reduced to 80% for large particles due to particle bounce. [32]

The glass-slide methods require multiple samples in several areas including uncontaminated areas in order to compare spore counts. Results are reported in concentrations of spores per cubic meter of air (spores/m^3). [5] [26] Analytical results may also report mold species, pollen, insect parts, and mycelial fragments collected.

Spore trap sampling does not allow the spore to be identified by species level, this is one of the disadvantages of using this method. Dirt, debris, and spores can also impact on the slide and mask the actual spore count or cause overloading.

A study was conducted to evaluate the collection efficiency of several spore trap samplers; Air-O-Cell, Burkard, and Button. The performance of the Air-O-Cell and Burkard personal samplers were very similar when the sample particles were larger than the cut off diameter. The Button sampler showed higher collection efficiency for spore sizes below 2.3 μm. The Button sampler

collection efficiency was the highest for spore sizes and was shown to be optimal for total bioaerosol sampling. However, the study concluded that the appropriate sampler to use depends on the reason for sampling, the environment, and the sampling conditions. [32]

1.3.6 Laser Particle Counters

Laser particle counters are direct reading instruments that use optical technology to detect particles from air, liquid, or a surface. Particle counters have three different classes: handheld, portable, or remote. Handheld counters are light, easy to use, and held by the operator. Portable counters are heavier and have more features than a handheld counter. They are placed in the area to be sampled, and the device is left in one location for the duration of the sample. Remote counters are used to calculate samples on a regular basis. They are fixed in the area and the data is sent to a control terminal.

Optical counters measure the light scattered by particles by refraction or diffraction. Laser particle counters are used in airborne contamination testing, clean-room application, aerospace industry, computer disc production, semiconductor industry, indoor air quality, and many other industries. These instruments will measure dust and biological contaminants such as mold, bacteria, viruses, animal dander, dust mites, pollen, human skin cells, cockroach and other insect parts. [33]

The Handheld 3016 IAQ particle counter is an optical counter that counts particles in varies size ranges (see Figure 11). Sensors inside the instrument measure the light scattered when a particle passes through the light beam. The number and size of the particle is measured by the strength of scattered light. The particle counter has six particle size channels and collects each channel concurrently. The Handheld particle counter has six channels: 0.3 μm, 0.5 μm, 1 μm, 2.5 μm, 5.0 μm, and 10 μm range. The Handheld particle counter has a flow rate of 2.83 L/min. Particles for each size channel are recorded and then converted into particles per cubic meter (p/m^3). [33]

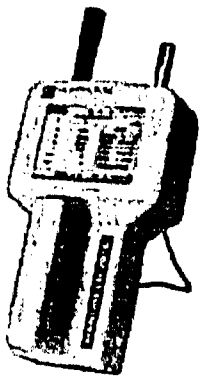

Figure 11: Handheld Direct Reading Particle Counter
Source: Lighthouse 2005

1.4 Mold Standards

There are no standards for mold, just guidelines for inspection, assessment, analysis and remediation of mold. Occupational Safety and Health Administration (OSHA) currently has no specific standard to address mold issues. OSHA does have specific indoor air quality issues covered under the

ventilation standard. Section 5(a)(1) of the OSHA Act, also known as the General Duty Clause, states that an employer must provide a workplace free from hazards. OSHA does have several documents and fact sheets that are available as guidelines to address mold issues in the home and work place. Most of the guidelines are shared by several organizations. [34] [35]

The Institute of Inspection, Cleaning, and Restoration Certification (IICRC) establishes education programs and oversees inspection, cleaning, and restoration in the service industry. They have established a standard for mold remediation (S520 Standard and Reference Guide for Mold Remediation). These guidelines are followed by those in the restoration industry to ensure proper cleanup of contaminated properties. [22] [23]

The New York City Guidelines were developed in 1991 to aid in remediation for Building Engineers and Managers servicing large office buildings and multi-family buildings. [22]

The Environmental Protection Agency (EPA) has two published guidelines, one for homeowners and one for schools and commercial buildings. The homeowner guidelines recommend personal protective equipment and also have recommendations for removal of mold. The other guidelines offer more comprehensive information on personal protective equipment, sampling, and removal of contamination for commercial buildings. [22] [36]

The American Conference of Governmental Industrial Hygienists (ACGIH) has many published resources available for inspection, assessment, sampling

and remediation. In addition to publications, ACGIH supports educational activities to exchange ideas, information and techniques for air sampling bioaerosols, and mold remediation. [36]

The American Industrial Hygiene Association (AIHA) has been involved with legislation covering issues related to mold in several states. AIHA believes individuals involved with inspection, assessment, analysis and remediation of mold need to be properly educated and trained in the occupational and environmental hazards of mold. An AIHA Mold Guideline on Assessment, Remediation, and Post-Remediation includes methodologies and techniques available to assess mold growth in residential and commercial buildings. Other publications AIHA is currently working on include "Field Guide for the Determination of Biological Contaminants in Environmental Samples" and the "Mold Green Book". There are several other organizations that offer guidelines on mold control and clean-up in the home and workplace. [23]

1.5 Sample Interpretation

When sampling for viable and non viable spores it is recommended to sample the outdoor environment for comparison. ACGIH, AIHA, EPA, Aerotech laboratories, and Environmental Microbiology Laboratory Inc. (EMLab) follow the same guidelines when interpreting sampling results. When sampling for viable or non-viable spores they recommend sampling the outdoor environment for a comparison. First a comparison is made between outdoor and indoor samples,

second a comparison is made between outdoor and indoor genera, and third, absence or presence of indicator/opportunistic organisms is determined.

When comparing outdoor vs. indoor samples, the indoor levels should be lower (30%-80%) than the outdoor levels. When using this comparison, the time of year and location must be taken into consideration. Spore counts are elevated in most locations in the spring and fall. In climates with high humidity outdoor spore counts may be high through most of the year. [4] [7] [8] [22] [23] [36]

In the spring and summer, counts inside a structure may be very similar to outdoor counts due to open windows, doors, and increased ventilation in the area. During the winter months outdoor counts are very low due to snow cover and may not provide a good comparison. If this is the case a sample may be taken in a "clean area" for comparison. [4] [7]

Comparing indoor and outdoor genera (spore type) must also be considered. The distribution of genera and species of the samples should be the same. If elevated counts of one spore type exist in the outdoor sample, then the indoor sample should also contain higher counts of the same species. Another indicator of a mold problem is the presence of indicator organisms such as *Stachybotrys chartarum*. [4] Stachybotrys requires significant moisture for a long period of time to sustain growth.

1.6 Objectives of Study

The purpose of this study was to determine if particle counts recorded with a direct reading particle counter (Lighthouse Handheld 3016) can be correlated with spore counts collected with a spore trap method (Air-O-Cell). If a correlation can be demonstrated, a direct reading particle counter may be used to identify problem areas and to determine if remediation has been effective.

1.7 Hypotheses

The following Hypotheses were developed from the objectives of this study:

1) He: There will be a correlation between particle counts collected with the Lighthouse Handheld 3016 IAQ direct reading particle counter and spore counts collected with an Air-O-Cell spore trap cassette inside the structure.

Ho: There will not be a correlation between particle counts collected with the Lighthouse Handheld 3016 IAQ direct reading particle counter and spore counts collected with an Air-O-Cell spore trap cassette inside the structure.

Decision Rule: A Pearsons correlation coefficient (cc) > .50, R-squared value > .50 and a P value < .05.

2) He: There will be a correlation between particle concentrations collected with the Lighthouse Handheld 3016 IAQ direct reading particle counter and spore concentrations collected with an Air-O-Cell spore trap cassette inside the structure.

Ho: There will not be a correlation between particle concentration collected with the Lighthouse Handheld 3016 IAQ direct reading particle counter and spore concentrations collected with an Air-O-Cell spore trap cassette inside the structure.

Decision Rule: A Pearsons correlation coefficient (cc) > .50, R-squared value > .50 and a P value < .05.

2.0 Materials and Methods

The data presented was collected using the Air-O-Cell Cassette and the Handheld 3016 particle counter. Both instruments were used simultaneously in the same location to determine if a correlation could be made between the data collected by the instruments.

2.1 Location

Twenty-nine samples were collected inside a cabin near Georgetown Lake in Southwestern Montana. For comparison purposes, one sample was collected outside of this structure. Sampling was conducted in December of 2004 with an

average outdoor temperature of 33˚F and an average relative humidity of 67%. The temperature inside the structure during the time of sampling averaged 45˚F with a relative humidity of 56%. The structure had a kitchen, frontroom, bathroom, and two bedrooms. The cabin had not been occupied for several years due to the mold damage inside the structure. A considerable amount of moisture made its way into the structure due to a broken water pipe under the cabin and possible water intrusion through the roof. Mold growth was present throughout the entire structure with visible mold on many surfaces including the ceiling, walls, carpet, furniture, and cabinets (see Figures 12 & 13). The ceiling and carpet had significant mold growth in several areas inside the cabin (see Figures 14 & 15). Several of the walls were covered with treated wood and had significantly less mold growth (see Figure 16).

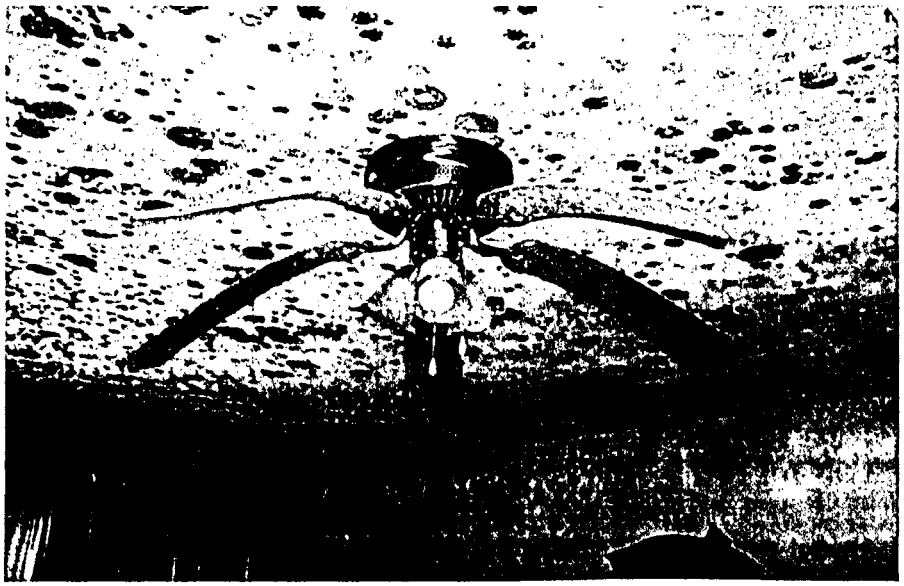

Figure 12: Visible mold growth on the frontroom ceiling

Figure 13: Visible mold growth on ceiling

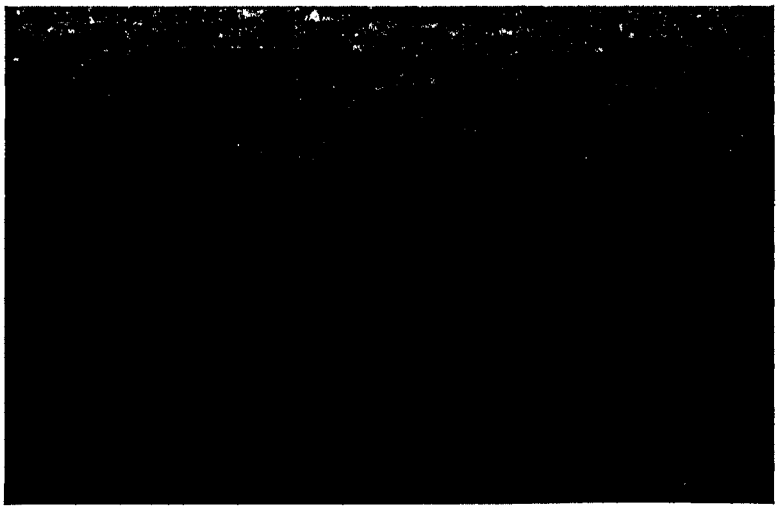

Figure 14: Visible mold growth on bedroom ceiling

Figure 15: Visible mold growth on ceiling

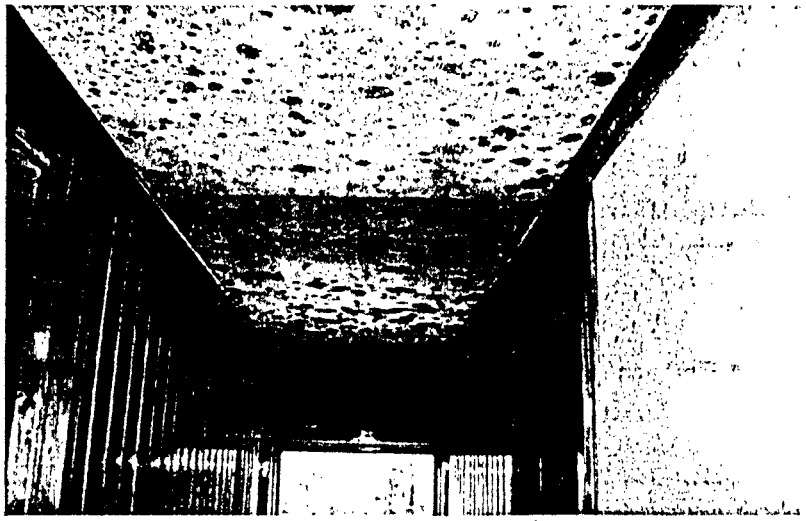

Figure 16: Less visible mold growth on treated wood

In addition to the mold contamination inside the structure, there was a significant amount of rodent debris (see Figure 17).

Figure 17: Rodent Contamination

2.2 Spore Trap Sampling

Thirty spore trap samples were collected throughout the structure using an Air-O-Cell Cassette and Zefon Mini-pump. The Zefon Mini pump was calibrated at 15L/min with a rotameter pre and post sampling. Samples were taken in the frontroom, bathroom, hallway, and both bedrooms. All samples were taken in the middle of each room with the cassette held at breathing zone height. Several samples were taken at each location and collected for varied lengths of time, 10 minutes, 5 minutes, and 2 minutes. Once the sample was drawn, the ends of the cassette were covered and then placed in clean plastic bags and sealed. Each cassette and bag was labeled to avoid sample location error.

2.3 Direct Reading Particle Counter

The Handheld 3016 IAQ Particle Counter was used simultaneously with the Air-O-Cell Cassette for all thirty samples. The particle counter recorded the relative humidity and temperature for every sample. Six different channel sizes were sampled; 0.3 μm, 0.1 μm, 1 μm, 2.5 μm, 5 μm, and 10 μm at 2.83 L/min. All six channel sizes were collected at the same time. The purge filter was used after each sample to zero the instrument. The location of each sample was entered into the instrument to correspond with the Air-O-Cell cassette. All samples were taken side-by-side with the corresponding Air-O-Cell samples. The particle counter was turned on at the same time as the Zefon pump and shut it self off at the end of the designated sample duration.

2.4 Sample Analysis

The Air-O-Cell cassettes were sent to Aerotech Laboratories for analysis. Aerotech Laboratories has many accreditations to ensure accuracy, reliability, and impartiality for its customers. Accreditations and quality programs are listed in Appendix A. Each cassette was opened at the lab prior to analysis. The sample trace was removed and lacto phenol cotton blue stain was placed on the slide. The entire trace or 100% of all samples were analyzed with light microscopy at 600X magnification. Both viable and non-viable spores were counted and reported as total counts or spores. This method does not allow for differentiation between Aspergillus and Penicillium therefore, they were grouped

together. Non-distinctive spores were reported in the ascospores or basidiospores categories. Genera with greater than 500 spores on one slide were reported as estimates.

3.0 Results and Discussion

3.1 Handheld 3016 IAQ Particle Counter Results

The Handheld particle counter results are divided by location and particle size. Cumulative and differential count data is reported by both particle count and particle concentration. The number of actual particles collected at each size channel is reported as particle counts. The concentration is calculated by the following equation:

$$\frac{\text{Counts}}{\text{FR X T/ 1000}} = \text{p/m}^3$$

Where:
Counts = particle counts
FR = flow rate in L/min
T = time in minutes

When calculating the concentration at each channel size the Differential data reports particle counts and particle concentration at six different cut points, not combining any two cut points. The raw data collected with the direct reading particle counter is included in Appendix B. The differential count and

concentration data is summarized in tables 1 & 2. Each figure indicates the average, maximum, minimum, and standard deviation.

Table 1: Differential Count Summary Recorded with the 3016 IAQ Particle Counter

Particle Cut Points Particle Count (p)	.3 μm (p)	.5 μm (p)	1 μm (p)	2.5 μm (p)	5 μm (p)	10 μm (p)
Average	197822.3	9098.6	22542.9	8342.3	9755.6	2805.5
Maximum	371401	16509	50592	20036	22990	6781
Minimum	68650	3867	2110	273	279	113
Standard Deviation	102833.9	3587.8	13900.3	5656.5	6804.8	1975.7

Table 2: Differential Concentration Summary Recorded with the 3016 IAQ Particle Counter

Particle Cut Points Particle Concentration p/m3	.3 μm p/m3	.5 μm p/m3	1 μm p/m3	2.5 μm p/m3	5 μm p/m3	10 μm p/m3
Average	16997137.2	824438.5	1908891.9	689092.7	793061.5	226660.1
Maximum	26231804.7	1166019.6	3573279.2	1415129.3	1623768.4	478937.5
Minimum	4848703.7	273123.6	149027.9	19281.8	19705.6	7981.1
Standard Deviation	5381841.3	219526.7	767903.6	319666.9	390017.3	116665.2

Cumulative data combines the counts from the highest cut point to the next lowest cut point. The last cut point 0.3 um is a total of all cut points. The cumulative count and concentration data, average, maximum, minimum, and standard deviation is summarized in tables 3 & 4.

Table 3: Cumulative Count Summary Recorded with the 3016 IAQ Particle Counter

Particle Cut Points Particle Count (p)	.3 μm (p)	.5 μm (p)	1 μm (p)	2.5 μm (p)	5 μm (p)	10 μm (p)
Average	250367.2	52545.0	43446.4	20903.5	12561.1	2805.5
Maximum	485592	114191	99722	49130	29094	6781
Minimum	88212	13902	2775	665	392	113
Standard Deviation	132624.8	31201.7	28119.2	14363.9	8748.7	1975.7

Table 4: Cumulative Concentration Summary Recorded with the 3016 IAQ Particle Counter

Particle Cut Points Particle Concentration (p/m3)	.3 μm p/m3	.5 μm p/m3	1 μm p/m3	2.5 μm p/m3	5 μm p/m3	10 μm p/m3
Average	21439281.9	4442144.8	3617706.2	1708814.3	1019721.6	226660.1
Maximum	34297038.8	8065234.1	7043298.3	3470019.1	2054889.8	478937.5
Minimum	6321042.7	981889.0	195996.4	46968.5	27686.7	7981.1
Standard Deviation	6987677.2	1696043.6	1563915.2	818730.2	503939.8	116665.2

The particle counts and concentration increase as the cut point decreases in size. Biological contaminations of indoor air include pollen, fungal spores, dust mites, animal dander, bacteria, and viruses. Particle sizes of these contaminants are illustrated in Table 5. [37]

Due to the rodent and animal activity inside the structure an increase in particle counts are expected.

Table 5: Particle Size of Indoor Biological Contaminants	
Pollen	10-100 microns
Fungal Spores	1-100 microns
Animal Dander	1-100 microns
Bacteria	0.2-2.0 microns
Viruses	0.01-0.3 microns
Estimation of biological contaminants size ranges vary for all biological contaminants (37)	

3.2 Spore Trap Air-O-Cell Data

The highest number of spore counts for species found in all Air-O-Cell cassette samples were Ascospores, Aspergillus/Penicillium-like, Basidospores, and Cladosporium; with Aspergillus/Penicillium-like comprising the bulk of the entire counts as illustrated in tables 6 & 7. Non distinctive spores that could not be classified were identified as ascospores or basidospores. They are classified as ascospores if produced in the ascus of the spore or as basidospores which includes mushrooms and other microfungi. Several other species were reported in lower concentrations depending on the location of the samples. Stachybotrys was detected in every location inside the structure. The highest concentration of Stachybotrys was located in the bathroom.

Table 6: Air-O-Cell Species Spore Counts by Species

Mold Species Spore Counts	Ascospores counts	Aspergillum/ Penicillium-like counts	Basidiospores counts	Cladopsorium counts	Stachybotrys counts
Average	54.9	17560.5	846.5	647.7	14.8
Maximum	174	35280	7501	2500	145
Minimum	6	32	8	7	0
Standard Deviation	45.9	8963.4	1341.7	530.8	28.7

Table 7: Air-O-Cell Species Spore Concentrations by Species

Mold Species Spore count concentration counts/m3	Ascospores counts/m3	Aspergillus/ Penicillium-like counts/m3	Basidiospores counts/m3	Cladopsorium counts/m3	Stachybotrys counts/m3
Average	943.4	281164.2	10834.7	9645.6	269.4
Maximum	5733.0	522667.0	50007.0	33333.0	2067.0
Minimum	107.0	427.0	107.0	93.0	0.0
Standard Deviation	1138.4	136866.5	9748.9	6742.1	557.7

A debris rating of 2 was given to all samples analyzed. The description of the rating indicates up to 25% of the slide was occluded with non-microbial particulates that can mask the presence of spores. Actual spore counts could be higher than the reported numbers.

The complete sample results for the Air-O-Cell cassettes are included in Appendix C. The first two samples collected were AS01 and AS02; these two samples were collected nine days prior to the other 28 samples. The particle counts collected with the handheld and spore counts collected with the Air-O-Cell were much lower for the first two samples than the other 28 samples. Several Air-O-Cell samples were collected in the same room within the structure and showed different counts and concentrations for the same location.

A study was conducted to determine if the time of day effects the outdoor concentrations. The study found the levels of fungi varied from the morning and afternoon samples at four different locations. This variation was independent of species, time of day, or location. The study concluded several outdoor samples at varying times of the day must be taken to get an adequate comparison for indoor contamination. [38]

3.3 Statistical Analysis

The particle concentration data collected from the Air-O-Cell Cassette and the Handheld Particle Counter was compiled into Minitab 14 ® version for statistical Analysis. The 2.5 μm cut point was used from the Handheld data for

comparison with the Air-O-Cell data (see Table 8). Mycelial fragments are separated from spore counts in the lab report, but were added into the total spore count for comparison with the handheld counts.

Table 8: Cumulative Data by Concentration (p/m3)

Sample Number / Sample Location	Air-O-Cell Results p/m3	Handheld Cumulative Results p/m3 for 2.5 μm Cutpoint
AS01/ Front room	79626	1214012
AS02/ Bedroom	82280	538690
AS03/ Front room	284680	625776
AS04/ Front room	481574	1531668
AS05/ Front room	371120	2307178
AS06/ Hallway	510707	2187179
AS07/ Hallway	287146	3075342
AS08/ Hallway	243440	1522203
AS09/ Hallway	252200	1122936
AS10/ Hallway	288534	866269
AS11/ Back Bedroom	307534	2286272
AS12/ Back Bedroom	384094	2161258
AS13/ Back Bedroom	389640	2124389
AS14/ Back Bedroom	331934	2270451
AS15/ Bedroom	485013	3048291
AS16/ Bedroom	353734	3470019
AS17/ Bedroom	288147	3209821
AS18/ Bathroom	275947	1801119
AS19/ Bathroom	238893	2210416
AS20/ Outside	760	46969
AS21/ Front room	209033	954555
AS22/ Front room	365766	921007
AS23/ Hallway	547333	1787275
AS24/ Hallway	431634	1536365
AS25/ Bathroom	529133	1332422
AS26/ Bathroom	432633	1665087
AS27/ Back bedroom	184240	1219769
AS28/ Back bedroom	105387	1360144
AS29/ Bedroom	150080	1447548
AS30/ Bedroom	226307	1420003

A basic regression was performed and three outliers were identified and removed. A moderate positive correlation (.536) was found between the Air-O-Cell spore concentrations and the handheld 2.5 um particle concentrations (p=.003) (see table 9). A weak R-squared value (.287) was also found (see table 9).

Table 9: Regression Analysis & Correlation Cumulative

Correlations: Air-O-Cell p/m^3, Handheld Cumulative p/m^3

Pearson correlation of Air-O-Cell p/m3 and Handheld Cumulative p/m3 = 0.536
P-Value = 0.003

Regression Analysis: Air-O-Cell p/m^3 versus Handheld Cumulative p/m^3

The regression equation is
Air-O-Cell p/m3 = 145839 + 0.0822 Handheld Cumulative p/m3

Predictor	Coef	SE Coef	T	P
Constant	145839	48487	3.01	0.006
Handheld	0.08222	0.02540	3.24	0.003

S = 111533 R-Sq = 28.7% R-Sq(adj) = 26.0%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	1.30400E+11	1.30400E+11	10.48	0.003
Residual Error	26	3.23430E+11	12439633205		
Total	27	4.53830E+11			

The particle count data collected from the Air-O-Cell cassette were compared to the cumulative 2.5 μm counts from the Handheld data (see Table 10).

Table 10:Cumulative Data Counts		
Sample Number/ Sample Location	Air-O-Cell Results Counts	Handheld Cumulative Results 2.5 μm Cutpoint
AS01/ Front room	11944	34377
AS02/ Bedroom	6171	7627
AS03/ Front room	21351	8860
AS04/ Front room	36118	21686
AS05/ Front room	27834	32666
AS06/ Hallway	38303	30967
AS07/ Hallway	21536	43542
AS08/ Hallway	18258	21552
AS09/ Hallway	18915	15899
AS10/ Hallway	21640	12265
AS11/ Back Bedroom	23065	32370
AS12/ Back Bedroom	28807	30600
AS13/ Back Bedroom	29223	30078
AS14/ Back Bedroom	24895	32146
AS15/ Bedroom	36376	43159
AS16/ Bedroom	26530	49130
AS17/ Bedroom	21611	45446
AS18/ Bathroom	20696	25501
AS19/ Bathroom	17917	31296
AS20/ Outside	57	665
AS21/ Front room	6271	5406
AS22/ Front room	10973	5216
AS23/ Hallway	16420	10122
AS24/ Hallway	12949	8701
AS25/ Bathroom	15874	7546
AS26/ Bathroom	12979	9430
AS27/ Back bedroom	13818	6908
AS28/ Back bedroom	7904	7703
AS29/ Bedroom	11256	8198
AS30/ Bedroom	16973	8042

A basic regression was performed and three statistical outliers were removed from the data. The removal of the outliers increased the R squared value by 15%. A strong positive correlation (.787) was found between the Air-O-Cell spore counts and the handheld 2.5um particle counts (p=.000) (see Table 11). A strong R-squared value (.619) was also found (see Table 11).

Table 11: Regression Analysis & Correlation Counts

Correlations: Air-O-Cell, Handheld Counts (C)

Pearson correlation of Air-O-Cell and Handheld Counts (C) = 0.787
P-Value = 0.000

Regression Analysis: Air-O-Cell versus Handheld Counts (C)

The regression equation is
Air-O-Cell = 9404 + 0.436 Handheld Counts (C)

Predictor	Coef	SE Coef	T	P
Constant	9404	1688	5.57	0.000
Handheld	0.43579	0.06833	6.38	0.000

S = 5148 R-Sq = 61.9% R-Sq(adj) = 60.4%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	1078342570	1078342570	40.68	0.000
Residual Error	25	662671019	26506841		
Total	26	1741013590			

Other statistical analysis was performed on the data to determine if any other correlations could be made. Data collected with the handheld and Air-O-Cell cassette in each room of the structure was separated and evaluated with

Minitab to see if a correlation could be made by room. No correlation was seen. All six cut points collected with the handheld particle counter were compared with the total spore count collected with the Air-O-Cell cassette. A very weak correlation was seen with all analyses excluding the 2.5 μm cut point.

4.0 Conclusion

The goal of this study was to determine if there was a correlation between spore counts collected with the Air-O-Cell spore trap cassette and particle counts collected with a Handheld direct reading particle counter. If a strong relationship could be identified between these sampling methods, it may prove to be valuable to those conducting indoor mold sampling in terms of both time and money. The direct reading particle counter results are obtained in real time, eliminating the wait for laboratory results. Intergrated sample analysis is costly and requires several samples to be analyzed in order to accurately evaluate an area.

In this study, thirty samples were taken with the Handheld 3016 IAQ Particle Counter and compared with the spore counts collected with the Air-O-Cell Cassette.

Several statistical analysis were performed. These analyses investigated the relationship between the integrated spore trap data and the handheld particle counter data. The particle counts and concentrations from all six size fractions, cumulative and differential, were included in this analysis. Only two

comparisons, particle concentrations, and particle count data from the Air-O-Cell and 2.5 μm handheld size fraction are discussed in this paper.

In the comparisons made between particle counts, a strong positive correlation coefficient was found (.787); and a strong R-squared value (.619) was noted (p = .000). Although an ideal correlation was not found, there was a significant relationship between particle counts as determined by the handheld particle counter and mold spores counts collected with the Air-O-Cell Cassette. Therefore, the research rejects the null of hypothesis #1.

Null Hypothesis #1:

There will not be a correlation between particle counts collected with the Lighthouse Handheld 3016 IAQ direct reading particle counter and spore counts collected with an Air-O-Cell spore trap cassette inside the structure.
Decision Rule: A Pearsons correlation coefficient (cc) > .50, R-squared value > .50, and a P value < .05.

When comparisons were made between particle concentrations; a positive moderate correlation coefficient was found (.536); however a weak R-squared value (.287) was noted (p=.003). Particle concentrations as determined by the handheld particle counter can not be used to predict the concentrations of mold spores collected with the Air-O-Cell Cassette. Therefore, the research failed to reject the null of hypothesis #2.

Null Hypothesis #2:

There will not be a correlation between particle concentrations collected with the Lighthouse Handheld 3016 IAQ direct reading particle counter and spore concentrations collected with an Air-O-Cell spore trap cassette inside the structure.

Decision Rule: A Pearsons correlation coefficient (cc) > .50, R squared value > .50, and a P value < .05.

4.1 Limitations of Research

There were several limitations of research in this study that must be noted. All samples were taken in the same relatively small structure making it difficult to extrapolate any conclusions to different sample locations. Several sample locations with varying degrees of contamination; no visible mold, minimal visible mold, and significant visible mold would have been a more representative sample.

The sample location was heavily contaminated with rodent droppings, urine, and debris. This could have drastically increased the particle counts in all size ranges recorded with the handheld counter. The handheld counter does not have the ability to differentiate between mold spores and other particles. The contamination also causes problems with sample analysis of the Air-O-Cell cassette. The debris can cover spores on the slide and underestimate the total spore count.

A study on fungal fragments present in contaminated environments was conducted using an aerosolization chamber. Aspergillus versicolor, Penicillium melinii, and Cladosporium cladosporioides were the fungal species used for this study. The study showed that fungal fragments are released from these species in higher numbers than intact spores. The release was not dependent on air velocity, surface material, or presence or absence of vibration. The study found that total fungal contamination in an area cannot be measured by counting only intact spores. The concentrations of fungal fragments are considerably higher

and must be considered to determine indoor air quality issues. [39] In this study, fungal fragments would have been counted by the handheld particle counter increasing the total particle count. The spore trap method would not count fungal fragments as spores but, may count a few as mycellial fragments.

The spore trap samples can be analyzed in different ways depending on which lab conducts the analysis. All labs do not use the same magnification and sample trace for analysis. The lab selected to perform the analysis in this study was Aerotech Laboratories. Aerotech uses 600 X magnification for spore identification and analyze 100% of the trace slide. Once 500 spores of the same species have been identified a statistical estimation is made for the rest of the slide. Another lab used for mold sampling, Environmental Microbial Labs (EM Labs) uses 100 X magnification for spore identification and analyzes 25% of the trace slide. The total spore count is then estimated from the rest of the trace slide. Therefore, it is difficult to compare spore counts analyzed from lab to lab. This may impact the reproducibility of this study due to cassette analysis.

In this study the highest correlation and R-squared values were noted with count comparisons. Comparisons of particle counts do not allow for differences in flowrates and sample volumes. The two sampling methods used in this study operated at different flow rates; the direct reading instrument required a flowrate of 2.83 L/min and the spore trap method utilized a pump with a flowrate of 15L/min.

Although significant relationships were noted between the direct reading and spore trap counts, these flow rate discrepancies may affect the reproductivity of this study.

5.0 Recommendation for Further Research

Indoor air quality continues to be an issue in many homes and workplaces due to several contaminants, including mold. The following recommendations can be considered for further research.

1. Increase the number of sample locations with varying degrees of mold contamination.
2. Conduct pre and post samples at contaminated structures that will be remediated.
3. Use more than one spore trap method for integrated sampling.

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Appendix A

Aerotech Laboratories Summary of Quality Programs

Summary of Quality Programs

The American Association for Laboratory Accreditation No. 2004-01
For technical competence in the field of Biological, Microbiological, and Biochemical Testing on Air, Bulk, Surface, Water, Wallcheck ™, Carpetcheck™, Dustcheck™, and Allergens.

American Industrial Hygiene Association EMLAP No. 102297
Industrial Hygiene Association's Environmental Microbiology Laboratory Accreditation Program Accredited to perform analysis of bacteria and fungi.

American Industrial Hygiene Assiciation EMPAT No. 102297
Industrial Hygiene Association's Environmental Microbiology Proficiency Analytical Testing Proficiency testing for indoor air quality microbiology

International Organization for Standardization ISO 17025
Demonstrated compliance with the ISO/IEC 17025 Standard

Certified Indoor Air Quality Professional 000240,000187,00236
Administered by The Association of Energy Engineers

FDA Registration No. 20110538
Registered for medical device manufacturing, contract sterilization and packaging, and good laboratory practices

Indoor Aero-Allergen Survey CAP No. 7140201-01
Administered by the College of American Pathologists

USDA Registraiton No. S-35038
Licensed to accept foreign soils for microbiological and chemical analysis

ADHS License No. AZ0611
Licensed for water, wastewater, and hazardous waste by the Environmental Protection Agency (EPA) through the Arizona Department of Health Services

Center for Disease Control and Prevention Laboratory Registration and Select Agent Transfer

Appendix B

Lighthouse Handheld 3016 IAQ Cumulative Particle Counter Data

Cumulative Handheld Particle Count Data

Timestamp	Location (Name)	0.3 micron (Counts)	0.5 micron (Counts)	1.0 micron (Counts)	2.5 micron (Counts)	5.0 micron (Counts)	10.0 micron (Counts)
12/20/2004 15:11:01	Location 001	248196	81411	72860	34377	20279	3918
12/20/2004 15:19:00	Location 002	89496	20846	16979	7627	4700	1152
12/29/2004 15:36:09	Location 003	177660	24518	17413	8860	5765	1633
12/29/2004 15:42:42	Location 004	276064	49133	40561	21686	13785	3225
12/29/2004 15:48:41	Location 005	371674	73724	63543	32666	19816	4375
12/29/2004 15:54:59	Location 006	358430	71250	60249	30967	19382	4725
12/29/2004 16:01:54	Location 007	445014	95310	81882	43542	27354	6781
12/29/2004 16:09:52	Location 008	280954	55754	45580	21552	12910	3036
12/29/2004 16:16:11	Location 009	222133	45329	36148	15899	9207	1918
12/29/2004 16:24:17	Location 010	189745	36335	27906	12265	7020	1405
12/29/2004 16:32:34	Location 011	371893	74859	64079	32370	20005	4801
12/29/2004 16:38:40	Location 012	365927	70995	60348	30600	19117	4528
12/29/2004 16:45:31	Location 013	368190	70240	59105	30078	18863	4805
12/29/2004 16:51:41	Location 014	378181	75622	63966	32146	20080	4828
12/29/2004 17:00:50	Location 015	432189	98055	85719	43159	25990	5589
12/29/2004 17:06:46	Location 016	485592	114191	99722	49130	29094	6104
12/29/2004 17:12:45	Location 017	457069	110391	94939	45446	26370	5201
12/29/2004 17:20:00	Location 018	324561	71984	57961	25501	14620	3128
12/29/2004 17:25:53	Location 019	376021	88387	71878	31296	17795	3521
12/29/2004 17:32:58	Location 020	145712	13902	2775	665	392	113
12/29/2004 17:37:10	Location 021	88212	18520	13696	5406	2837	504
12/29/2004 17:39:42	Location 022	88531	17523	12850	5216	2794	504
12/29/2004 17:42:45	Location 023	138338	26462	20942	10122	6207	1401
12/29/2004 17:45:44	Location 024	126495	24707	19194	8701	5135	1137
12/29/2004 17:48:53	Location 025	116143	24074	18090	7546	4365	993
12/29/2004 17:52:09	Location 026	135167	28156	21615	9430	5519	1273
12/29/2004 17:55:46	Location 027	104170	21494	16743	6908	3841	782
12/29/2004 17:58:37	Location 028	115460	23580	18245	7703	4341	915
12/29/2004	Location	116272	24780	19208	8198	4727	996

18:02:09	029						
12/29/2004	Location	117528	24817	19196	8042	4524	874
18:04:30	030						
	Average	250367.2	52545.0	43446.4	20903.5	12561.1	2805.5
	Maximum	485592	114191	99722	49130	29094	6781
	Minimum	88212	13902	2775	665	392	113
	Standard Deviation	132624.8	31201.7	28119.2	14363.9	8748.7	1975.7

Cumulative Handheld Particle Count Data Average, Maximum, Minimum, and Standard Deviation

Cumulative Handheld Particle Count Data

0.3 micron (p/m^3)	0.5 micron (p/m^3)	1.0 micron (p/m^3)	2.5 micron (p/m^3)	5.0 micron (p/m^3)	10.0 micron (p/m^3)
8764958.9	2875002.3	2573026.6	1214012.3	716146.1	138362.9
6321042.7	1472339.1	1199215.4	538689.9	331957.9	81365.0
12548007.2	1731690.0	1229868.6	625775.9	407178.1	115337.7
19498216.0	3470231.0	2864796.4	1531667.7	973625.3	227779.6
26251086.5	5207076.9	4487999.7	2307177.8	1399590.9	309003.3
25315671.6	5032339.9	4255346.6	2187178.5	1368937.7	333723.6
31431041.7	6731681.7	5783271.0	3075342.4	1931994.8	478937.5
19843593.5	3937867.8	3219285.0	1522203.4	911824.7	214430.7
15689105.5	3201557.0	2553109.1	1122935.8	650284.3	135467.1
13401562.7	2566316.8	1970982.1	866268.8	495817.9	99234.2
26266554.3	5287241.2	4525857.0	2286271.5	1412939.8	339091.4
25845179.7	5014329.5	4262339.0	2161257.6	1350220.9	319809.6
26005013.9	4961004.3	4174546.7	2124389.1	1332281.1	339373.9
26710671.6	5341131.4	4517875.9	2270450.5	1418237.0	340998.4
30525220.5	6925559.2	6054275.7	3048291.4	1835656.3	394747.3
34297038.8	8065234.1	7043298.3	3470019.1	2054889.8	431121.4
32282478.3	7796842.6	6705478.2	3209820.6	1862495.5	367343.2
22923526.7	5084181.9	4093746.7	1801118.6	1032600.8	220928.6
26558112.2	6242714.8	5076695.2	2210415.6	1256849.0	248685.9
10291541.3	981889.0	195996.4	46968.5	27686.7	7981.1
15575886.7	3270138.1	2418348.3	954555.4	500938.5	88993.0
15632213.6	3094094.5	2268967.3	921006.5	493345.9	88993.0
24426801.5	4672483.5	3697798.7	1787275.3	1095990.7	247379.2
22335643.5	4362597.3	3389148.5	1536364.6	906704.1	200763.9
20507756.4	4250826.4	3194211.6	1332422.4	770742.6	175337.3
23866887.4	4971598.7	3816632.5	1665086.5	974508.2	224777.9
18393643.9	3795267.2	2956367.3	1219768.6	678218.2	138080.3
20387156.8	4163599.1	3221580.4	1360144.4	766504.8	161564.6
20530534.3	4375487.1	3391620.5	1447548.2	834662.1	175867.0
20752310.4	4382020.4	3389501.7	1420002.7	798817.7	154325.1
21439281.9	4442144.8	3617706.2	1708814.3	1019721.6	226660.1
34297038.8	8065234.1	7043298.3	3470019.1	2054889.8	478937.5
6321042.7	981889.0	195996.4	46968.5	27686.7	7981.1
6987677.2	1696043.6	1563915.2	818730.2	503939.8	116665.2

Cumulative Handheld Particle Count Data

Sample Time (m)	Sample Volume (L)	Temperature (F)	Relative Humidity (%)
10.000	28.3	39.3	67.7
5.000	14.2	39.8	56.3
5.000	14.2	46.3	44.9
5.000	14.2	46.3	46.4
5.000	14.2	46.8	46.4
5.000	14.2	47.3	51.1
5.000	14.2	47.7	50.6
5.000	14.2	47.3	50.9
5.000	14.2	47.3	50.8
5.000	14.2	46.8	48.7
5.000	14.2	46.3	50.3
5.000	14.2	44.9	50.4
5.000	14.2	45.4	52.3
5.000	14.2	45.4	53.1
5.000	14.2	45.4	54.8
5.000	14.2	44.9	56.9
5.000	14.2	44.9	57.5
5.000	14.2	45.4	61.6
5.000	14.2	45.4	61.4
5.000	14.2	33.7	67.0
2.000	5.7	40.2	66.3
2.000	5.7	42.1	62.1
2.000	5.7	43.1	57.7
2.000	5.7	44.0	57.4
2.000	5.7	44.5	59.4
2.000	5.7	44.5	61.6
2.000	5.7	44.5	59.2
2.000	5.7	44.9	58.9
2.000	5.7	44.5	56.7
2.000	5.7	44.0	58.2
4.167	11.8	44.4	55.9
10.000	28.3	47.7	67.7
2.000	5.7	33.7	44.9
1.802	5.1	2.9	6.1

Appendix C

Aerotech Laboratory Inc. Analytical Reports

Aerotech Laboratory Inc. Analytical Reports

Sample Number	1				2			
Sample Identification	AS01 Front Room				AS02 Bedroom			
Date Analyzed	12/28/2004				12/28/2004			
Volume(M^3)	0.1500				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	3				2			
Analyte	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%
Mycelial Fragments	32	213	7	n/a	16	213	13	n/a
Pollen	<1	<7	7	n/a	<1	<13	13	n/a
Total Fungal Spores	11,912	79,413	7	100	6,155	82,067	13	100
	Fungal Spore Identification				Fungal Spore Identification			
Alternaria	1	7	7	<1				
Arthrinium								
Ascospores	88	587	7	<1	10	133	13	<1
Aspergillus/Penicillium- Like	3,500	23,333	7	29	4,000	53,333	13	65
Basidiospores	7,501	50,007	7	63	2,000	26,667	13	32
Bipolaris/Drechslera								
Botrytis								
Chaetomium								
Cladosporium	800	5,333	7	7	143	1,907	13	2
Curvularia								
Epicoccum								
Fusarium								
Memnoniella								
Nigrospora								
Oidium/Peronospora								
Pithomyces	1	7	7	<1				
Rusts								
Smuts/Myxomycetes/Periconia	1	7	7	<1				
Stachybotrys	1	7	7	<1				
Stemphylium	1	7	7	<1				
Torula								
Ulocladium	2	13	7	<1				
Unclassified Conidia	16	107	7	<1	2	27	13	<1
Data Qualifier	D102				D102			

Sample Number	1				2				3				4			
Sample Identification	AS03 Front Room				AS04 Front Room				AS05 Front Room				AS06 Hallway			
Date Analyzed	1/21/2005				1/21/2005				1/21/2005				1/21/2005			
Volume(M^3)	0.0750				0.0750				0.0750				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	2				2				2				2			
Analyte	Total Count	Count/M^3		%	Total Count	Count/M^3		%	Total Count	Count/M^3		%	Total Count	Count/M^3		%
		Result	DL			Result	DL			Result	DL			Result	DL	
Mycelial Fragments	9	120	13	n/a	98	1,307	13	n/a	37	493	13	n/a	236	3,147	13	n/a
Pollen	1	13	13	n/a	1	13	13	n/a	<1	<13	13	n/a	1	13	13	n/a
Total Fungal Spores	21,342	284,560	13	100	36,020	480,267	13	100	27,797	370,627	13	100	38,067	507,560	13	100
	Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification			
Alternaria					1	13	13	<1	6	80	13	<1	1	13	13	<1
Arthrinium																
Ascospores	19	253	13	<1	174	2,320	13	<1	59	787	13	<1	104	1,387	13	<1
Aspergillus/Penicillium- Like	20,720	276,267	13	97	34,160	455,467	13	95	25,760	343,467	13	93	33,600	448,000	13	88
Basidiospores	31	413	13	<1	680	9,067	13	2	840	11,200	13	3	1,700	22,667	13	4
Bipolaris/Drechslera																
Botrytis					2	27	13	<1	3	40	13	<1	7	93	13	<1
Chaetomium																
Cladosporium	570	7,600	13	3	990	13,200	13	3	1,100	14,667	13	4	2,500	33,333	13	7
Curvularia																
Epicoccum																
Fusarium																
Memnoniella																
Nigrospora																
Oidium/Peronospora																
Pithomyces																
Rusts													1	13	13	<1
Smuts/Myxomycetes/Periconia	1	13	13	<1					1	13	13	<1	2	27	13	<1
Stachybotrys									3	40	13	<1	10	133	13	<1
Stemphylium																
Torula																
Ulocladium	1	13	13	<1	13	173	13	<1	25	333	13	<1	142	1,893	13	<1
Unclassified Conidia																
Data Qualifier	A95				A95				A95				A85			

Sample Number	5				6				7				8			
Sample Identification	AS07 Hallway				AS08 Hallway				AS09 Hallway				AS10 Hallway			
Date Analyzed	1/21/2005				1/21/2005				1/21/2005				1/21/2005			
Volume(M^3)	0.0750				0.0750				0.0750				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	2				2				2				2			
Analyte	Total Count	Count/M^3		%	Total Count	Count/M^3		%	Total Count	Count/M^3		%	Total Count	Count/M^3		%
		Result	DL			Result	DL			Result	DL			Result	DL	
Mycelial Fragments	31	413	13	n/a	18	240	13	n/a	19	253	13	n/a	101	1,347	13	n/a
Pollen	<1	<13	13	n/a	<1	<13	13	n/a	1	13	13	n/a	1	13	13	n/a
Total Fungal Spores	21,505	286,733	13	100	18,240	243,200	13	100	18,896	251,947	13	100	21,539	287,187	13	100
	Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification			
Alternaria	2	27	13	<1	5	67	13	<1	2	27	13	<1				
Arthrinium																
Ascospores	47	627	13	<1	62	827	13	<1	46	613	13	<1	56	747	13	<1
Aspergillus/Penicillium- Like	20,160	268,800	13	94	16,240	216,533	13	89	16,800	224,000	13	89	20,160	268,800	13	94
Basidiospores	354	4,720	13	2	600	8,000	13	3	810	10,800	13	4	500	6,667	13	2
Bipolaris/Drechslera																
Botrytis	9	120	13	<1	5	67	13	<1	3	40	13	<1	4	53	13	<1
Chaetomium	1	13	13	<1									1	13	13	<1
Cladosporium	910	12,133	13	4	1,300	17,333	13	7	1,200	16,000	13	6	800	10,667	13	4
Curvularia																
Epicoccum													1	13	13	<1
Fusarium																
Memnoniella																
Nigrospora																
Oidium/Peronospora													1	13	13	<1
Pithomyces	2	27	13	<1												
Rusts									2	27	13	<1				
Smuts/Myxomycetes/Periconia	2	27	13	<1					1	13	13	<1	5	67	13	<1
Stachybotrys					3	40	13	<1	2	27	13	<1	5	67	13	<1
Stemphylium																
Torula																
Ulocladium	18	240	13	<1	25	333	13	<1	28	373	13	<1	6	80	13	<1
Unclassified Conidia									2	27	13	<1				
Data Qualifier	A95				A95				A95				A95			

Sample Number	9				10				11				12			
Sample Identification	AS11 Back Bedroom				AS12 Back Bedroom				AS13 Back Bedroom				AS014 Back Bedroom			
Date Analyzed	1/21/2005				1/21/2005				1/21/2005				1/21/2005			
Volume(M^3)	0.0750				0.0750				0.0750				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	2				2				2				2			
Analyte	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%
Mycelial Fragments	17	227	13	n/a	92	1,227	13	n/a	75	1,000	13	n/a	74	987	13	n/a
Pollen	1	13	13	n/a	1	13	13	n/a	2	27	13	n/a	2	27	13	n/a
Total Fungal Spores	23,048	307,307	13	100	28,715	382,867	13	100	29,148	388,640	13	100	24,821	330,947	13	100
	Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification			
Alternaria									9	120	13	<1	1	13	13	<1
Arthrinium																
Ascospores	60	800	13	<1	11	147	13	<1	35	467	13	<1	71	947	13	<1
Aspergillus/Penicillium- Like	21,280	283,733	13	92	26,320	350,933	13	92	27,440	365,867	13	94	22,960	306,133	13	93
Basidiospores	580	7,733	13	3	1,401	18,680	13	5	810	10,800	13	3	980	13,067	13	4
Bipolaris/Drechslera																
Botrytis	2	27	13	<1	6	80	13	<1	4	53	13	<1	1	13	13	<1
Chaetomium									2	27	13	<1	1	13	13	<1
Cladosporium	1,100	14,667	13	5	920	12,267	13	3	800	10,667	13	3	800	10,667	13	3
Curvularia																
Epicoccum																
Fusarium																
Hemoniella																
Nigrospora																
Oidium/Peronospora																
Pithomyces	5	67	13	<1	10	133	13	<1	7	93	13	<1	1	13	13	<1
Rusts	2	27	13	<1	1	13	13	<1	1	13	13	<1				
Smuts/Myxomycetes/Periconia	4	53	13	<1	15	200	13	<1	7	93	13	<1				
Stachybotrys	8	107	13	<1	16	213	13	<1	23	307	13	<1	2	27	13	<1
Stemphylium																
Torula																
Ulocladium	7	93	13	<1	13	173	13	<1	9	120	13	<1	1	13	13	<1
Unclassified Conidia					2	27	13	<1	1	13	13	<1	3	40	13	<1
Data Qualifier	A95				A95				A95				A95			

Sample Number	9				10				11				12			
Sample Identification	AS11 Back Bedroom				AS12 Back Bedroom				AS13 Back Bedroom				AS014 Back Bedroom			
Date Analyzed	1/21/2005				1/21/2005				1/21/2005				1/21/2005			
Volume(M^3)	0.0750				0.0750				0.0750				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	2				2				2				2			
Analyte	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%
Mycelial Fragments	17	227	13	n/a	92	1,227	13	n/a	75	1,000	13	n/a	74	987	13	n/a
Pollen	1	13	13	n/a	1	13	13	n/a	2	27	13	n/a	2	27	13	n/a
Total Fungal Spores	23,048	307,307	13	100	28,715	382,867	13	100	29,148	388,640	13	100	24,821	330,947	13	100
	Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification			
Alternaria									9	120	13	<1	1	13	13	<1
Arthrinium																
Ascospores	60	800	13	<1	11	147	13	<1	35	467	13	<1	71	947	13	<1
Aspergillus/Penicillium- Like	21,280	283,733	13	92	26,320	350,933	13	92	27,440	365,867	13	94	22,960	306,133	13	93
Basidiospores	580	7,733	13	3	1,401	18,680	13	5	810	10,800	13	3	980	13,067	13	4
Bipolaris/Drechslera																
Botrytis	2	27	13	<1	6	80	13	<1	4	53	13	<1	1	13	13	<1
Chaetomium									2	27	13	<1	1	13	13	<1
Cladosporium	1,100	14,667	13	5	920	12,267	13	3	800	10,667	13	3	800	10,667	13	3
Curvularia																
Epicoccum																
Fusarium																
Memnoniella																
Nigrospora																
Oidium/Peronospora																
Pithomyces	5	67	13	<1	10	133	13	<1	7	93	13	<1	1	13	13	<1
Rusts	2	27	13	<1	1	13	13	<1	1	13	13	<1				
Smuts/Myxomycetes/Periconia	4	53	13	<1	15	200	13	<1	7	93	13	<1				
Stachybotrys	8	107	13	<1	16	213	13	<1	23	307	13	<1	2	27	13	<1
Stemphylium																
Torula																
Ulocladium	7	93	13	<1	13	173	13	<1	9	120	13	<1	1	13	13	<1
Unclassified Conidia					2	27	13	<1	1	13	13	<1	3	40	13	<1
Data Qualifier	A85				A85				A95				A85			

Sample Number	13				14				15				16			
Sample Identification	AS15 Front Bedroom				AS16 Front Bedroom				AS17 Front Bedroom				AS18 Bathroom			
Date Analyzed	1/21/2005				1/21/2005				1/21/2005				1/21/2005			
Volume(M³)	0.0750				0.0750				0.0750				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	2				2				2				2			
Analyte	Total Count	Count/M³ Result	DL	%	Total Count	Count/M³ Result	DL	%	Total Count	Count/M³ Result	DL	%	Total Count	Count/M³ Result	DL	%
Mycelial Fragments	144	1,920	13	n/a	71	947	13	n/a	69	920	13	n/a	65	867	13	n/a
Pollen	<1	<13	13	n/a	2	27	13	n/a	<1	<13	13	n/a	<1	<13	13	n/a
Total Fungal Spores	36,232	483,093	13	100	26,459	352,787	13	100	21,542	287,227	13	100	20,631	275,080	13	100
	Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification			
Alternaria	1	13	13	<1	1	13	13	<1					4	53	13	<1
Arthrinium																
Ascospores	24	320	13	<1	15	200	13	<1	31	413	13	<1	124	1,653	13	<1
Aspergillus/Penicillium- Like	35,280	470,400	13	97	24,640	328,533	13	93	19,600	261,333	13	91	19,040	253,867	13	92
Basidiospores	600	8,000	13	2	1,100	14,667	13	4	600	8,000	13	3	540	7,200	13	3
Bipolaris/Drechslera																
Botrytis					7	93	13	<1					22	293	13	<1
Chaetomium													13	173	13	<1
Cladosporium	309	4,120	13	<1	670	8,933	13	3	1,300	17,333	13	6	660	8,800	13	3
Curvularia																
Epicoccum																
Fusarium																
Memnoniella																
Nigrospora																
Oidium/Peronospora																
Pithomyces	4	53	13	<1	8	107	13	<1	3	40	13	<1	44	587	13	<1
Rusts					1	13	13	<1								
Smuts/Myxomycetes/Periconia	2	27	13	<1	6	80	13	<1	1	13	13	<1	4	53	13	<1
Stachybotrys	2	27	13	<1	5	67	13	<1	5	67	13	<1	145	1,933	13	<1
Stemphylium																
Torula																
Ulocladium	10	133	13	<1	5	67	13	<1	2	27	13	<1	35	467	13	<1
Unclassified Conida					1	13	13	<1								
Data Qualifier	A85				A85				A85				A85			

Sample Number	17				18				19				20			
Sample Identification	AS19 Bathroom				AS20 Outside				AS21 Front Room				AS22 Front Room			
Date Analyzed	1/21/2005				1/21/2005				1/21/2005				1/21/2005			
Volume(M^3)	0.0750				0.0750				0.0750				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	2				2				2				2			
Analyte	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%
Mycelial Fragments	75	1,000	13	n/a	2	27	13	n/a	6	80	13	n/a	4	53	13	n/a
Pollen	<1	<13	13	n/a	<1	<13	13	n/a	<1	<13	13	n/a	1	13	13	n/a
Total Fungal Spores	17,842	237,893	13	100	55	733	13	100	6,255	83,533	13	100	10,969	146,253	13	100
	Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification			
Alternaria	1	13	13	<1												
Arthrinium																
Ascospores	112	1,493	13	<1	8	107	13	15	6	80	13	<1	8	107	13	<1
Aspergillus/Penicillium- Like	16,800	224,000	13	94	32	427	13	58	5,973	79,640	13	95	10,500	140,000	13	96
Basidiospores	259	3,453	13	1	8	107	13	15	124	1,653	13	2	132	1,760	13	1
Bipolaris/Drechslera																
Botrytis	6	80	13	<1												
Chaetomium	14	187	13	<1					1	13	13	<1				
Cladosporium	630	8,400	13	4	7	93	13	13	158	2,107	13	3	326	4,347	13	3
Curvularia																
Epicoccum																
Fusarium																
Memnoniella																
Nigrospora																
Oidium/Peronospora																
Pithomyces																
Rusts																
Smuts/Myxomycetes/Periconia	3	40	13	<1					1	13	13	<1	1	13	13	<1
Stachybotrys	16	213	13	<1					2	27	13	<1	2	27	13	<1
Stemphylium																
Torula																
Ulocladium	1	13	13	<1												
Unclassified Conidia																
Data Qualifier	A85				A85				A85				A85			

Sample Number	21				22				23				24			
Sample Identification	AS23 Hallway				AS24 Hallway				AS25 Bathroom				AS25 Bathroom			
Date Analyzed	1/21/2005				1/21/2005				1/21/2005				1/21/2005			
Volume (M^3)	0.0750				0.0750				0.0750				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	2				2				2				2			
Analyte	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%	Total Count	Count/M^3 Result	DL	%
Mycelial Fragments	18	240	13	n/a	2	27	13	n/a	25	333	13	n/a	21	280	13	n/a
Pollen	<1	<13	13	n/a	<1	<13	13	n/a	<1	<13	13	n/a	<1	<13	13	n/a
Total Fungal Spores	16,402	219,693	13	100	12,947	172,627	13	100	15,849	211,320	13	100	12,958	172,773	13	100
	Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification			
Alternaria	1	13	13	<1	1	13	13	<1	1	13	13	<1	1	13	13	<1
Arthrinium																
Ascospores	24	320	13	<1	41	547	13	<1	96	1,280	13	<1	172	2,293	13	1
Aspergillus/Penicillium- Like	15,680	209,067	13	96	12,040	160,533	13	93	15,120	201,600	13	95	12,390	165,200	13	96
Basidiospores	600	8,000	13	4	359	4,787	13	3	402	5,360	13	3	206	2,747	13	2
Bipolaris/Drechslera																
Botrytis									10	133	13	<1	3	40	13	<1
Chaetomium	2	27	13	<1					4	53	13	<1	4	53	13	<1
Cladosporium	51	680	13	<1	500	6,667	13	4	152	2,027	13	<1	114	1,520	13	<1
Curvularia																
Epicoccum																
Fusarium																
Memnoniella																
Nigrospora																
Oidium/Peronospora																
Pithomyces	7	93	13	<1	2	27	13	<1	6	80	13	<1	2	27	13	<1
Rusts	1	13	13	<1												
Smuts/Myxomycetes/Periconia					2	27	13	<1	6	80	13	<1	2	27	13	<1
Stachybotrys	8	107	13	<1	2	27	13	<1	49	653	13	<1	62	827	13	<1
Stemphylium																
Torula																
Ulocladium	29	373	13	<1					3	40	13	<1	2	27	13	<1
Unclassified Conidia																
Data Qualifier	A85				A85				A85				A85			

Sample Number	25				26				27				28			
Sample Identification	AS27 Back Bedroom				AS28 Back Bedroom				AS29 Front Bedroom				AS30 Front Bedroom			
Date Analyzed	1/21/2005				1/21/2005				1/21/2005				1/21/2005			
Volume (M³)	0.0750				0.0750				0.0750				0.0750			
Percent Of Trace Analyzed	100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification				100% of Trace at 600x Magnification			
Debris Rating	2				2				2				2			
Analyte	Total Count	Count/M³		%	Total Count	Count/M³		%	Total Count	Count/M³		%	Total Count	Count/M³		%
		Result	DL			Result	DL			Result	DL			Result	DL	
Mycelial Fragments	8	107	13	n/a	2	27	13	n/a	25	333	13	n/a	14	187	13	n/a
Pollen	<1	<13	13	n/a	<1	<13	13	n/a	1	13	13	n/a	<1	<13	13	n/a
Total Fungal Spores	13,810	184,133	13	100	7,902	105,360	13	100	11,231	149,747	13	100	16,959	226,120	13	100
	Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification				Fungal Spore Identification			
Alternaria																
Arthrinium																
Ascospores	60	800	13	<1	31	413	13	<1	41	547	13	<1	11	147	13	<1
Aspergillus/Penicillium- Like	12,950	172,667	13	94	7,490	99,867	13	95	10,500	140,000	13	93	15,680	209,067	13	92
Basidiospores	184	2,453	13	1	123	1,640	13	2	500	6,667	13	4	870	11,600	13	5
Bipolaris/Drechslera																
Botrytis	2	27	13	<1					2	27	13	<1	3	40	13	<1
Chaetomium					1	13	13	<1	1	13	13	<1	1	13	13	<1
Cladosporium	580	7,733	13	4	246	3,280	13	3	174	2,320	13	2	341	4,547	13	2
Curvularia																
Epicoccum																
Fusarium																
Memnoniella																
Nigrospora																
Oidium/Peronospora																
Pithomyces	2	27	13	<1	1	13	13	<1	3	40	13	<1	24	320	13	<1
Rusts													2	27	13	<1
Smuts/Myxomycetes/Periconia					5	67	13	<1	4	53	13	<1	2	27	13	<1
Stachybotrys	32	427	13	<1	2	27	13	<1	4	53	13	<1	10	133	13	<1
Stemphylium																
Torula																
Ulocladium					3	40	13	<1	2	27	13	<1	15	200	13	<1
Unclassified Conidia																
Data Qualifier	A85				A85				A85				A85			

Indoor Environmental Report

For

Riley, Tiffany

Project Name: Center Lode

Project Number:

Laboratory Number: 915-501-2090

AIHA
Environmental
Microbiology
ACCREDITED
LABORATORY

ACCREDITED

Purpose

To characterize environmental samples collected by Riley, Tiffany and received by Aerotech P&K on
01/20/2005 for microbiological contaminants as directed.

Introduction

Mold, also known as fungi, are microscopic organisms that can be found virtually everywhere, indoors
and outdoors. In the presence of excess moisture, molds can grow rapidly to produce adverse conditions. In response to increasing public concern, a number of authorities, including the United States EPA, California Department of Health Services and New York City Department of Health, have
developed recommendations and guidelines for assessment and remediation of mold. Websites for
these organizations can be found at the end of this report.

While it is generally accepted that molds can be allergenic, infectious and toxic, there are no generally
accepted numerical guidelines for interpretation of microbial data. The absence of standards makes
interpretation of microbial data somewhat challenging. This report has been designed to provide some basic interpretive information using certain assumptions and facts that have been extracted from a number of authoritative bodies and peer reviewed text, such as the American Conference of
Governmental Industrial Hygienists (ACGIH). In the absence of standards, the user must determine
the appropriateness and applicability of this report to the given situation. Identification of the presence
of a particular fungus in an indoor environment does not necessarily mean that the building occupants
are or are not being exposed to antigenic or toxic agents.

None of the information contained herein should be construed as medical advice or a call to action for
evacuation or remediation. Any decision relative to medical significance should be made by a qualified physician.

Aerotech Laboratories did not conduct a site investigation, provide consulting or collect samples referenced in this report. Aerotech's sole involvement in this project is to provide analytical results for
samples submitted. The data presented in this report are based on the samples and accompanying
information provided and represents concentrations at a point in time under the conditions sampled.

All aspects of this report are governed by Aerotech's standard terms and conditions.

Materials

1. Samples collected by Riley, Tiffany. Project Name: Center Iode. Please reference chain of custody included with this report.

Methods

1. Surface and Bulk Samples

Bulk, swab, and dust samples undergo an aqueous extraction and subsequent microscopic analysis. Tape samples are analyzed directly; spores are counted and characterized. All

samples are analyzed via light microscopy at 600X magnification. The results are reported as total, meaning they include both viable and non-viable fungal spores. This technique does not allow for the differentiation between *Aspergillus* and *Penicillium* spores. Additionally, depending on morphology, other non-distinctive spores will be reported in categories such as ascospores (produced in an ascus) or basidiospores (borne outside a basidium including the mushrooms and other microfungi).

2. Air Samples – Spore Trap Device

Spore traps are a unique sampling device designed for the rapid collection and analysis of a wide range of airborne particulates, including fungal spores. Samples are analyzed via light microscopy at 600X magnification, with the entire trace (100% of the sample) being analyzed . The results are reported as total, meaning they include both viable and non-viable fungal spores. This technique does not allow for the differentiation between *Aspergillus* and *Penicillium* spores. Additionally, depending on morphology, other non-distinctive spores will be reported in categories such as ascospores (produced in an ascus) or basidiospores (including the mushrooms and other microfungi). Genera with greater than 500 spores on a slide are difficult to count and are therefore estimations. Similarly, excessive non-microbial particulates can mask the presence of fungal spores, thereby reducing counting accuracies. All slides are graded with the following debris scale for data qualification.

Debris Rating Scale

Non-Microbial Particulate Debris	Description	Interpretation
0	No particles detected.	No particulates on slide. The absence of particulates could indicate improper sampling, as most air samples typically contain some particulates.
1	Minimal non-microbial debris present.	Reported values are not affected by debris.
2	Up to 25% of the slide occluded with non-microbial particulates.	Non-microbial particulates can mask the presence of fungal spores. As a result, actual values could be higher than the numbers reported. Higher debris ratings increase the probability of this bias.
3	26% to 75% of the slide occluded with non-microbial particulates.	
4	76% to 90% of the slide occluded with non-microbial particulates.	
5	Greater than 90% of the slide occluded with non-microbial particulates.	*Air-O-Cell or LARO Cassettes - Sample could not be read due to excessive debris. Reported concentrations are estimations calculated from the number of spores observed on the perimeter of debris. The sample should be collected at shorter time interval, or other measures taken to reduce the collection of non-microbial debris. *Other Cassettes - Sample could not be read due to excessive debris. The sample should be collected at shorter time interval, or other measures taken to reduce the

3. Data Qualifiers

The *Data Qualifiers* identify issues or events that are relevant to your analytical results. A data qualifier includes information about the validity, the source of the data whether calculated, entered or estimated, and the value of an observation. If applicable, a key is attached to each report type. In each case the data qualifiers provide significant information vital to the interpretation of the laboratory data.

4. Data Interpretation

According to ACGIH, "Data from individual sampling episodes is often interpreted with respect to baseline data from other environments or the same environment under anticipated low exposure conditions." In the absence of established acceptable exposure limits, it is often necessary to use a comparison standard when interpreting data. In this instance it will be necessary to sample the suspect area as well as a non-suspect area.

According to ACGIH, "...active fungal growth in indoor environments is inappropriate and may lead to exposure and adverse health effects."

a. Total Fungal Spores

According to ACGIH, ".... differences that can detected with manageable sample sizes are likely to be in 10-fold multiplicative steps (e.g., 100 versus 1000...)". Following this logic, if total fungal spores are ten (10) times greater in the sample from a suspect area than in the negative control sample collected from a non-suspect area, then that sample area may be a fungal amplification site.

b. Mycelial Fragments

Mycelium is a fungal mass that constitutes the vegetative or living body of a fungus . Following the same logic above, if total mycelial fragments are ten (10) times greater in the suspect sample than in the negative control, then the sample area is considered to be a fungal amplification site. The presence of mycelial fragments provides evidence of microbial growth.

c. Toxic Molds

Certain authorities refer to certain molds as important toxigenic taxa. The presence of a few spores of toxic mold should be interpreted with caution. Additionally, it should be recognized that these named molds are not necessarily the only ones of potential significance.

d. Water Indicator Molds

Certain authorities identify certain molds whose presence indicates excessive moisture. The presence of a few spores of indicator mold should be interpreted with caution. Additionally, it should be recognized that these named molds are not necessarily the only ones of potential significance.

e. Mold Glossary

Specific characteristics of the individual molds listed in the report are presented in Table I.

f. Useful Resources

i. Guidelines on Assessment and Remediation of Fungi in Indoor Environments, New York City Department of Health

Table I: Summary of Specific Mold Characteristics

Fungi	Environmental Indicator		Growth Indoors
Alternaria			Alternaria can grow indoors on a variety of substances.
Arthrinuim			*Arthrinium* is a widespread fungus found on plants. It is rarely found growing indoors.
Ascospores			Ascospore is a general classification for spores produced by sexual reproduction and can include *Aspergillus, Penicillium*, and *Ascotrica*. Frequently found growing on damp substrates.
Aspergillus/Penicillium-like			*Aspergillus* and *Penicillium* spores are indistinguishable via direct microscopic examination. *Aspergillus* tends to colonize continuously damp materials such as damp wallboard and fabrics. *Penicillium* is commonly found in house dust, on water-damaged wallpaper, behind paint and in decaying fabrics.
Aureobasidium			*Aureobasidium* is commonly found in a variety of soils. Indoors, it is commonly found where moisture accumulates, especially bathrooms and kitchens, on shower curtains, tile grout, windowsills, textiles, and liquid waste materials.
Basidiospores			Basidiospore is a general classification of spore that is commonly found in gardens, forests and woodlands. They are also agents of dry, white and brown rot.
Bipolaris/Dreschlera			*Bipolaris* and *Drechslera* can grow on a variety of substrates.
Botrytis			A mold that can be found associated with indoor plants.
Chaetomium			*Chaetomium* can be commonly found on damp sheetrock paper.
Cladosporium			*Cladosporium* is a common outdoor mold that can colonize continuously damp materials such as damp wallboard and fabrics.
Curvularia			*Curvularia* can grow on a variety of substrates.
Epicoccum			*Epicoccum tends to colonize continuously damp materials such as damp wallboard and fabrics.*
Fusarium			*Fusarium* colonize continuously wet materials such as soaked wallboard and water reservoirs for humidifiers and drip pans.
Memnoniella			*Memnoniella* can be found growing on a variety of cellulose-containing materials.
Nigrospora			*Nigrospora* is rarely found growing indoors.
Oidium/Peronospora			Both of these organisms are plant pathogens and cannot grow on indoor surfaces.
Pithomyces/Ulocladium			*Pithomyces* are rarely found indoors. *Ulocladium* colonize continuously damp materials such as wallboard and fabrics.
Rusts			Rusts are plant pathogens and only grow on host plants.
Smuts/Myxomycetes			Smuts do not usually grow indoors. They are parasitic plant pathogens that require a living host. Myxomycetes are occasionally found indoors.
Stachybotrys			*Stachybotrys* colonizes continuously wet materials such as soaked wallboard and water reservoirs for humidifiers and drip pans.
Stemphylium			*Stemphylium* is rarely found growing indoors.
Torula			*Torula* can grow indoors on cellulose containing materials.
Unidentified Conidia			An uncharacteristic fungal spore that does not lend itself to classification via direct microscopy.

 -Potential Toxigenic Mold

 -Potential Water Indicator Mold

Quality Programs

Aerotech Laboratories is staffed with over 200 professionals, including PhD's, chemists, and registered microbiologists with over 40 years of experience. The reliability of test results depends on
many factors such as the personnel performing the tests, environmental conditions, selection and validation of test methods, equipment functioning, measurement traceability, as well as the sampling,
storage and handling or test items, all of which are a reflection of the laboratories overall quality system.

Aerotech Laboratories, Inc. has modeled its quality system after ISO 17025 guidelines, one of the most stringent sets of standards in the industry, to ensure that its customers receive the high standard
of accuracy, reliability, and impar t lal l ty that they have come to expect f rom a leader in the environmental industry. Aerotech Laboratories' adherence to the standards set forth in the ISO 17025
guidelines has been validated and formally recognized through accreditations granted by two independent outside agencies, the American Industrial Hygiene Association (AIHA), and the American
Association for Laboratory Accreditation (A2LA). As an additional measure to demonstrate its competency to perform the analyses it offers to its clients, Aerotech Laboratories also participates in a
variety of different proficiency testing programs, including the Environmental Proficiency Analytical
Testing Program (EMPAT) sponsored by the American Industrial Hygiene Association.
As part of its continuous commitment to excellence, Aerotech Laboratories is also inspected, licensed
and/or accredited by a number of governmental agencies and independent associations in addition to
those already mentioned above. The scope document, accreditation certificates, and proficiency results can all be accessed at www.aerotechlabs.com. Below you will find additional information regarding the specific analyses requested for this project.