



Report Supporting Document

ANALYSIS OF FUNGI (MOLD) IN THE INDOOR ENVIRONMENTS BY DIRECT MICROSCOPIC EXAMINATION

(Analysis Code: FDE)

INTRODUCTION

Fungi are essential components of ecosystems and widely distributed in the nature. Fungal spores may be easily dispersed into indoor environments, and if conditions are suitable, some fungal spores may germinate and grow indoors. Mold, a common term for certain filamentous fungi, is fast growing and capable of producing hundreds of thousands of spores in a short period of time. Mold spores germinate and rapidly reproduce under suitable moisture, temperature, and nutrient conditions, and disperse through air turbulence, water drops, or insect activities. Mold has been associated with a number of adverse health effects, such as allergies, irritations, infections, and toxic reactions. In certain circumstances, monitoring or investigating indoor mold population is an important and essential step toward establishing a cause-and-effect relationship to health complaints.

Although there are general guidelines available for mold investigation, each project has special conditions that should be taken into consideration. The connection between mold exposure and certain illnesses is hard to establish. Since there is no widely accepted standard on the mold exposure limit, factoring in special conditions becomes even more important and illustrates the importance of proper sampling, accurate data analysis and appropriate interpretation of laboratory results.

Aemtek, Inc. is committed to providing fast, accurate and reliable analytical data based on samples and information submitted. As an environmental testing laboratory, Aemtek's role is to provide analytical data to facilitate microbiological investigations. Aemtek does not conduct sample collection, site evaluation or consultation. Interpretation of the data presented in this report should best be left to the primary investigator.

The purpose of this report is to present scientific data obtained by analyzing the samples submitted to Aemtek, Inc. for evaluation of fungal contamination in an indoor environment.

MATERIALS AND METHODS

As a part of investigation project, samples were collected and transported to the Aemtek microbiology laboratory for analysis. Data on sample identification, location, and air volume (for air samples) on this report are from the original **Chain of Custody Form**. Aemtek preserves the analytical preparations of the samples for **30 days** after the issuing of this report, after which time they will be sterilized and discarded.

Fungal Direct Examination

The samples were prepared in the laboratory for observation using a Phase Contrast Microscope with 150X – 1500X magnifications. The microscope is regularly aligned and calibrated. Lacto-cotton blue, distilled and sterile water, or when necessary, emission oil was used to prepare the microscopic slides. Taxonomic references, especially monographs and peer-reviewed publications, were used to guide identifications of fungi. Fungi are identified to the best taxonomic level allowed by the morphological characteristics present in the sample.

Air samples (Method ID: Aemtek SOP AF101)

Air sampling using spore trapping device has the advantage of providing volumetric data. An air sample cassette consists of a microscope slide that traps aerosols, biological or non-biological, as a measured volume of air is pulled through the cassette. This sampling method captures both viable and non-viable spores, which are expressed as total fungal spores.

The slide from the cassette is observed directly under a phase contrast microscope at 600x magnification. To ensure accuracy, Aemtek analyzes 100% of the deposition trace. Air volume and spore count are used to calculate number of spores per cubic meter of air. If a spore type is extremely abundant, it is counted up to **200** spores, then the total number is calculated based on the number of spores counted and the percentage of trace examined to reach that number. Examination continues for other spore types. The percentage of each type of spores, out of total spores, is given to evidence the distribution of spore types and to indicate the predominant spore type, if any. Data of pollen and insect/dust mite body parts are also provided to give baseline information of the bio-aerosol composition.

General density is an estimated percentage of area covered by all airborne particulates and can be used as an indicator of the “cleanness” or “background noise”. 0% indicates a blank sample; and 1-50% is typically proper for spore counting, although there is still a possibility that spores may be masked by non-biological particulates. High density (51%-99% = “dirty” or “noisy”) may result in underestimation due to the masking of fungal spores. Since it is impossible to give a realistic count of fungal spores, overloaded samples (general density $\geq 100\%$) will be rejected, unless analysis is specifically requested by the client.

Calculations

The calculation of particle concentration per cubic meter of air can be performed by using the following formulae.

- a. Determine the actual air volume collected in cubic meters (m^3) by following Formula A.

Formula A:

$$\text{Air volume (m}^3\text{)} = (\text{Sampling rate (liters per minute)} \times \text{Number of minutes}) / 1000$$

- b. Determine the length of sample trace counted based on the microscope field of view and number of fields of view counted. Accurately calibrate and measure the diameter of the microscope field of view using a stage micrometer slide. The analyst should record the number of complete traverses examined across the width of the deposition trace and use Formula B to calculate the actual length of the deposition trace examined.

Formula B:

$$\text{Trace Length Counted (mm)} = \text{Microscope field diameter (mm)} \times \text{number of traverses}$$

c. The concentrations of particles (cts/m³) can then be determined by using Formula C.

Formula C:

$$\text{Cts/m}^3 = \frac{\text{Trace length (14.4mm)}}{\text{Total length of trace counted (From Formula B)}} \times \frac{1}{\text{Air Volume (m}^3\text{) (From Formula A)}} \times \text{\# of particle counts}$$

Example 1: Fungal spore counted for the entire trace

If:

$$\text{Sample volume (15 lpm @ 5 minutes)} = (15 \times 5)/1000 = 0.075 \text{ m}^3$$

$$\text{Fungal spore counts} = 80$$

Then:

$$\text{Spore concentration} = \frac{1}{0.075 \text{ m}^3} \times 80 = 1,040 \text{ Cts/m}^3$$

Example 2: Fungal spore counted to 220 after 10 complete traverses

If:

$$\text{Microscope field diameter at 600X} = 0.350 \text{ mm}$$

$$\text{Number of traverses} = 10$$

$$\text{Sample volume (15 lpm @ 5 minutes)} = (15 \times 5)/1000 = 0.075 \text{ m}^3$$

$$\text{Fungal spore counts} = 220$$

Then:

$$\text{Spore concentration} = \frac{14.4 \text{ mm}}{0.350 \text{ mm} \times 10} \times \frac{1}{0.075 \text{ m}^3} \times 220 = 11,660 \text{ Cts/m}^3$$

Limit of Detection

The Limit of Detection (LOD) refers to the smallest concentration of an analyte that can be detected by the measurement system.

For the *Air-O-Cell* sample, the particle must be present on the slide to be detected. If the entire trace is analyzed, the smallest spore count is 1. The LOD is one spore or fungal fragment X per unit air sampled.

For example, if 75 L (=0.075 m³) of air is sampled, then

$$\text{Limit of Detection} = 1/0.075 \text{ m}^3 \times 1 \text{ spore count} = 13 \text{ spore counts/m}^3$$

Surface Samples - Bulk, Dust, Swab, Tape (Method ID: Aemtek SOP AF102)

Surface sampling is primarily used to detect mold growth or confirm the existence of mold colonies at the sampling site. Surface sampling is also occasionally used to obtain spore distribution data to determine if mold is growing indoors, but in a different site. Direct microscopic examination of bulk, dust, swab, and tape samples shows if mold colonies are present and the spore type and number.

Surface samples are prepared for microscopic examination using slides or clear tapes. Spores are identified and semi-quantified. Identification is performed to the best level possible without culturing. If multiple fruiting structures and abundant spores are observed, the existence of fungal colony is indicated on the report.

Limit of Detection (LOD) of direct microscopy is determined to be one fungal spore, hyphal fragment, or other structure, but due to the nature of the sample, the results are best presented in a semi-quantitative way.

RESULTS

The results of the laboratory analysis are shown on the **Data Sheet**. Spore identifications are presented as spore type, genus, or species when possible. Only when necessary, the **Analyst's Comments** sheet is provided to complement the data sheet by providing descriptive information that are not shown on the data sheet, notes on the analytical results, or other messages that the analyst wishes to convey to the client. Information on spore morphology, ecology, potential health effects, and secondary metabolite production of commonly encountered fungi are compiled by Aemtek analysts and can be provided upon request.

DISCUSSION

Hyphal fragments

Hypha is the vegetative unit of fungi. If the number of hyphal fragments is substantially greater in an indoor sample than in a control/outdoor sample, or if there exist a large number of hyphal fragments alone, it maybe an indication of indoor mold growth. Although a hyphal fragment is not a fungal spore, it can be dispersed and grow into an individual fungus when conditions are suitable. Therefore, fungal hyphal fragments are reported here with the spores.

Indoor vs. outdoor spore counts

Normally, indoor spores are a subset of outdoor spores in both type and number. In general, indoor spore levels range from 0-80% of an outdoor spore count. A substantial increase of a particular spore type in an indoor sample that is inconsistent with an outdoor sample, or a large number of a fungal species in an indoor sample but not existent in an outdoor sample, is often an indication of indoor mold growth.

Investigative sampling

In areas where amplification of mold is suspected, or there is a suspicion that health complaints are possibly caused by excessive mold exposure, it is advised that at least three areas be sampled:

1. Suspected/concerned area
2. Indoor unconcerned area, to serve as a control

3. An outdoor sample, for comparison

It is also recommended that due to each method's limitations, a combination of culturable and non-viable samples from the same area be used. If indoor mold amplification is suspected, both air samples and tape/bulk/swab samples be taken to locate/verify the source of contamination.

Outdoor spores found in indoor environment

Each fungus has its own natural habitat and ways of dispersal. Knowledge of fungal ecology is helpful for understanding laboratory results. Certain fungi are distinct outdoor organisms - their presence in indoor environments is most likely an influx from outdoors such as rusts, smuts, basidiospores, and some ascospores, *etc.* These fungi associate with plants as parasites, pathogens, or mycorrhizae, *etc.* Typical indoor molds can make use of food or other materials inside buildings as substrates, such as *Aspergillus*, *Chaetomium*, *Cladosporium*, *Penicillium*, *Stachybotrys*, *Ulocladium*, among others.

Spore counts on surface sample

The primary purpose of surface sampling is to detect mold growth at the sampling site or a nearby area. We do semi-quantitative analysis on surface samples. Presence of fungal spores/hyphae on the sample is characterized into several categories. The actual spore counting is not appropriate or necessary for fungal direct examination of surface samples because of limitations of both sample matrixes and analysis type requested. When interpreting report, one should pay attention to indications of colonies, spore types of unusually large number, or presence of moisture indicator fungi, such as *Alternaria*, *Chaetomium*, *Memmoniella*, *Stachybotrys*, *Ulocladium*, *etc.*

Interpretation of Data

Laboratory data should be considered within the scope of the sampling design to demonstrate the magnitude of indoor contamination problem, therefore, interpretation of laboratory data is best left to the primary consultant. Some publications offer guidelines for data interpretation, e.g., AIHA's *Field Guide for the Determination of Biological Contaminants in Environmental Samples*. The principles of data interpretation include recognizing typical outdoor fungi, dominant species, presence of potential pathogenic or toxic fungi in indoor environment, presence of moisture indicator fungi, *etc.*

ABOUT AEMTEK

Aemtek, Inc. is an environmental microbiology laboratory serving the indoor air quality industry. Its mission is to provide accurate, fast, and reliable expert services for detection, identification, analysis of fungi and bacteria in human environments. Aemtek also provides contracted research and expert testimony services. Aemtek is committed to the highest level of quality, scientific integrity, and customer service.

Aemtek, Inc. implements a series of quality assurance procedures. All analytical protocols are developed based on scientific merits and are validated regularly. Aemtek is accredited by the American Industrial Hygiene Association (AIHA) Environmental Microbiology Laboratory Accreditation Program (Lab No.: 167620).

ABOUT THE ANALYSTS

Florence Q. Wu, Ph.D.

Dr. Florence Wu earned a Ph.D. degree in mycology from the University of Tennessee in 1991, and has vast experience in fungal identification, taxonomy, biodiversity, and biogeography. Dr. Wu was a principal investigator of a National Science Foundation research project on fungal biodiversity. She has published many scientific papers and is an active member of several professional organizations. She specializes in identifying fungi using microscopic examination, culture methods, and DNA techniques.

Steven Y. Huang, Ph.D.

Dr. Steven Huang received a Ph.D. degree in plant pathology focused on forest mycology and microbiology. Before joining Aemtek, Dr. Huang was an associate professor in the Institute of Microbiology, CAS, a visiting scholar in the International Mycological Institute (United Kingdom) and Duke University, and a research associate in the University of Pittsburgh. His expertise includes microbial isolation, recovery, and identification, fungal biodiversity, PCR and DNA sequencing techniques.

Kuldeep K. Johal, Ph.D.

Dr. Johal earned her Ph.D. degree in mycology (taxonomy) in 2004. She has over five years mycological research experience, during which she collected various fungal species and found many new geographic records of fungi. She has published two scientific papers based on her findings. She joined Aemtek, Inc. in February 2004 and is currently an analyst in mycology.

CONTACT INFORMATION

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