



Report Supporting Document

ANALYSIS OF FUNGI (MOLD) IN INDOOR ENVIRONMENTS USING FUNGI CULTURABLE METHODS

(Analysis Codes: FCG, FCS, FCS/A, FCS/P)

INTRODUCTION

Fungi are essential components of ecosystems and widely distributed in 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. 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.

As an environmental testing laboratory, Aemtek's role in the project 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**.

Fungi Culturable Method

The fungi culturable method normally involves preparation of culture, incubation, observation, and identification. For species identification, it is often necessary to use subculture or slide culture technique. Culture preparation method varies among sample types (see below for detail).

Mature fungal colony was observed macroscopically and then prepared for microscopic observation using either direct examination of a portion of the colony or adhesive tape technique. Microscope examination was performed using a Phase Contrast Microscope with 150X – 1500X magnifications. The microscope is regularly aligned and calibrated. Lacto-cotton blue, distilled and sterile water, or sometimes, stain was used to prepare the microscopic slides. Taxonomic references, especially monographs and peer-reviewed publications, were used to guide fungal identifications. Fungi were identified to the taxonomic level (e.g., genus or species) requested by the client or to a characteristic group (e.g., yeasts, sterile mycelium), as allowed by the morphological characteristics present in the culture.

Fungal identification to species level is highly complicated and requires education, experience and use of taxonomic references. Identification characteristics vary greatly according to genus. Isolation, culture and identification methods in selected reliable references are followed, including those included in Klich (2002) for *Aspergillus*; Pitt (2000) for *Penicillium*, de Hoog et al. (2000) for hyphomycetes fungi with clinical significance; and Samson et al. (2000) for food- and airborne fungi.

Air samples (Method ID: Aemtek SOP AF103)

Air sampling, for example, using Anderson N6 sampler, has the advantage of providing volumetric data. A Petri dish containing culture medium was used to capture aerosols, biological or non-biological, as a measured volume of air is pulled in. The Petri dish with the sample was then incubated after received by the laboratory. Fungal spores and hyphal fragments (together called propagules) that germinated and grew on the culture medium were identified and counted as CFU (=Colony Forming Units). By factoring in the air volume sample, this sampling analysis generated quantitative data on viable and culturable fungi. Laboratory Limit of Detection (LOD) is determined to be one CFU.

Surface Samples – Bulk, Culture (e.g., contact plate), Dust, Swab (Method ID: Aemtek SOP AF104)

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.

An initial check was performed to evaluate the condition of the sample and to determine the recovery strategies. Direct plating was performed for samples with low count in addition to serial dilution plating. Serial dilution method was used to obtain propagule suspension series after the sample was washed thoroughly with sterile and distilled water. A measured volume from each propagule suspension was poured and spread onto a pre-selected culture medium and subsequently incubated to obtain fungal colonies suitable for identification. Plates with optimal number of countable colonies were selected for further analysis. Sample weight or area sampled, concentration of propagule suspension, and number of CFU were used to calculate final concentration of CFU.

Culture media and growth condition

Two media, MEA and DG18, were used routinely, and if necessary, a third media such as cellulose agar was also used to bring out certain fungi. Malt extract agar (MEA) is a wide spectrum medium for recovering many fungi, Dichloran glycerol agar (DG18) is for xerophilic saprotrophic fungi, and cellulose agar is for cellulotic fungi, such as *Stachybotrys*. Another medium, CYA, may be used for identification of *Aspergillus* and *Penicillium* species as specified in standard reference methods.

Fungal cultures were normally incubated at 25°C and for 7 days before identification were attempted. Higher incubation temperature (35°C) may be adapted to aid identification of thermophilic

fungi, such as *Aspergillus fumigatus*. Longer incubation time is needed to induce fungal sporulation or if subculturing was performed.

RESULTS

The results of the laboratory analysis are shown on the **Data Sheet**. Fungal identification results are presented as colony forming unit (CFU) of the genus or species identified. Results of air sample analysis is shown as CFU/m³, bulk and dust sample as CFU/mg, and swab sample as CFU/Swab or CFU/1000cm². If client prefers, CFU/Sample will be used. The percentage of each fungal taxon (taxonomic unit, such as genus and species) present in the sample is also given on the Data Sheet. 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, reported health effects, and secondary metabolite production of commonly encountered fungi are compiled by the Aemtek analysts and will be provided upon request.

DISCUSSION

Viable, culturable, and total fungi

Viable and culturable refer to different groups, i.e., viable organisms may not be culturable on the culture medium or growth condition specified. In contrast to air sampling of total fungal spores, air sampler impacting aerosols on culture medium provide information on culturable propagules only, which are a portion of viable fungi and an even smaller portion of total fungi. Sampling of culturable fungi can greatly underestimate the total propagule numbers due to several factors: 1) viable but non-culturable microorganisms, such as obligate parasites that cannot grow on media, 2) decline in fungal spore viability, 3) culture medium selection, 4) competition among microorganisms, 5) difference in growth rate of fungi, 6) damage to the propagules during sampling, 7) growth conditions, and 8) sampler performance.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection is the minimum concentration of viable aerosols in air that can be detected. According to AIHA's *Field Guide for the Determination of Biological Contaminants in Environmental Samples* (AIHA Press, 1996), the predicted LOD for Andersen impactor sampling at a rate of 28.3L/min for 5 min, is 10 CFU/m³. Limit of Quantitation is estimated as 30 times the LOD, following the same conditions. Such the LOQ for Andersen impactor sampling under the same condition is 300 CFU/m³.

The laboratory Limit of Detection (LOD) is one CFU.

Subcultures and slide cultures

Subculture refers to inoculation of original colony onto new culture medium to isolate a fungus of interest and to promote sporulation. Slide culture involves placing a glass slide cover onto an inoculum to capture sporulating structures and spores for examination under the microscope. The methods are often used for species level identification.

Indoor vs. outdoor CFU counts

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

Investigative sampling

Sampling strategy applied to total spore count can also be applied to culturable fungi 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 bulk/dust/swab samples be taken to locate/verify the source of contamination.

Culturable fungi on surface samples

The primary purpose of surface sampling is to detect mold growth at the sampling site or a nearby area. When interpreting report, one should pay attention the presence of unusually large number, or presence of moisture indicator fungi, such as *Alternaria*, *Chaetomium*, *Memnoniella*, *Stachybotrys*, *Ulocladium*, etc. As in air samples, fungi culturable method only detects fungi that grow on the specified culture media and growth conditions, such it is highly possible that underestimation occurs.

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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