ENVIRONMENTAL ANALAYISIS ASSOCIATES, INC.
BIOAEROSOL ANALYSIS METHOD GUIDE #2
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Analysis: Analysis of Surface Dust & Mold
Sampling Method: Transparent Tape / Bio-tape

Daniel M. Baxter

Two basic procedures are provided by EAA for the analysis of surface dust and mold spores. Both of the following procedures are designed for samples collected using transparent tape. The qualitative procedure is provided for the analysis of mold spores only. The quantitative procedure provides analysis of mold spores and other settled dust particles.

The EAA methods are based on modification of airborne spore counting criteria established for the evaluation of airborne pollen and mold spores utilizing slit impaction devices. Since regulatory requirements and accepted industry standards are not currently available, data obtained by these sampling and analysis procedures should be utilized for comparative purposes and to assist in the location and/or identification of particulate contamination sources. High surface concentrations of any particulate contaminant do not necessarily imply that a hazard exists.

1.0 Summary of The Tape Sampling And Analysis Method

Samples are collected using transparent tape by pressing the tape on the surface to be tested (one time only) and then reapplying the tape to a glass microscope slide for transportation to the laboratory.

In the laboratory, the tape is carefully removed from the slide, turned over with the adhesive side facing up and affixed to a new slide with one drop of acetone. One drop of Lacto-Phenol Cotton Blue stain is placed on the tape surface and a cover slip is placed on top.

Slides are examined utilizing bright field microscopy at a magnification of 400-900X. Identification of bioaerosols and other dust particles are performed by comparison with photographic references and known standards.

2.0 Qualitative Mold Spore Analysis

The qualitative surface mold analysis procedure is specifically designed to determine the presence or absence of mold spores (only) on building surfaces, and whether evidence of surface growth is present or absent. No quantitative calculation of surface concentration is performed. No analysis of other bioaerosols or classification of unusual particles is reported, and this procedure should be employed only when mold contamination is the sole bioaerosol or particle of interest.

The qualitative method will determine the following conditions:

1. Whether or not dust or a discolored or water-stained surface is inorganic dust or construction debris.

2. When significant quantities of mold spores have settled on a surface, and; if the mold on the surface indicates Agrowth≅, i.e. the presence of significant mold spores attached to mycelia fragments or other growth structures.

Data given in the report includes the following:

1. Relative concentration of other (non-mold related) surface dust (none, low, moderate, medium).

2. The presence or absence of mold spores and their classification by genus and species when possible.

3. Qualitative estimate of surface concentration (none, low, moderate, medium).

4. Evidence of mycelia growth (none, low, moderate, medium)

When more comprehensive dust analysis information is required, the Aquantitative≅ procedure described below should be utilized.

3.0 Quantitative Dust and Mold Spore Analysis

The semi-quantitative surface dust analysis procedure is designed for the analysis of common bioaerosols and airborne dust contaminants including mold, pollen, fibers, paint particles, dust and other aerosols. This analysis quantifies surface dust particle concentrations per square millimeter of surface area and determines if there are potentially elevated conditions. The semi-quantitative procedure provides the following:

1). Relative concentration of surface dust particle concentrations (none, low, moderate, high).

2). The presence or absence of mold spores and their classification by genus and species when possible.

3). Quantitative estimate of surface mold spore concentrations (spores per square millimeter of surface)

4). Quantitative estimate of mycelia growth (mycelia fragments per square millimeter of surface).

5). Quantitative estimate of other aerosols including skin cell fragments, cellulosic fibers, fiberglass fibers, opaque particles, insect parts, or other unusual particles.

3.1 Interpretation of Analysis Results:

With appropriate information, results from these analysis procedures can indicate when abnormal conditions are present whether from mold growth, malfunctioning HVAC equipment, or lack of maintenance or housekeeping. The results are divided into 6 different

particle classifications including:

1. Mold spores
2. Algal or fern spores
3. Pollen
4. Skin cell fragments (dander)
5. Fiberglass fibers
6. Insect parts
7. Opaque particles

3.1.1 Interpretation of Mold Spore Concentrations (Quantitative)

With appropriate historical information on the surfaces being tested, analysis results can often determine when heavy spore deposition by gravity settling is present, when mold growth is present, and sporulating. The analysis format provides a descriptive comments section describing the general condition the sample represents, and a quantitative calculation of mold spore or particle concentrations on building surfaces.

Mold spores, pollen, algae, other spores, skin cell fragments (dander), fibers, and opaque particles (paint, combustion emissions) are quantified as counts per square millimeter (cts/mm^2). These concentrations are to be utilized as reference for the purpose of categorizing surface contamination. The guidelines given in Table 3 are useful in making recommendations regarding the relative level of contamination, potential origin, potential duration of saturation, and remediation recommendations. These guidelines are not intended as a direct indicator of hazard, nor do these imply that a regulatory standard for surface contamination exists.

Table 1. General Interpretation Guidelines For Mold Spores

Spore Counts / mm^2		Description
#<10		Normal office building or ?clean≅ home
10-50	(total spores)	Dusty office or home
10 -50	(any one species)	Potential deposition from a nearby active growth
50-100	(Total or any one species)	Potential nearby source
50-100	(mycelia growth present)	Low mold growth
> 100	and mycelia present	Mold growth

3.1.2 Interpretation of Mold Growth Patterns in Buildings

A succession of mold species over time may occur on building materials once they have become saturated with water. Some mold species such as Penicillium and Aspergillus[4] are “primary” colonizers, grow quickly, and are the first molds to appear and colonize water saturated cellulose containing materials such as boxes, drywall paper, and ceiling tiles. Growth of these molds can typically be sustained after an initial saturation period followed by sustained relative humidity of 80% or greater. Primary colonizing molds will appear on exposed interior surfaces more readily than secondary or tertiary colonizers. Secondary colonizers such as Cladosporium, followed by tertiary colonizers including Ulocladium,

Aspergillus niger, Chaetomium, and Stachybotrys, will appear when repeated and chronic moisture is present, and near-surface relative humidity approaching 95% is sustained over several weeks. Secondary and tertiary species are most likely to grow in confined spaces such as wall cavities and behind baseboards and are less visible and are often overlooked during indoor air quality investigations. Some common indicator species for the range of moisture conditions described above are given in the following table. Growth conditions can vary significantly depending on the initial spore species present, physical condition and porosity of the substrate, source of moisture (i.e. water break, soil flood, sewer backflow), temperature, humidity, and degree of air movement surrounding the substrate. General material growth characteristics for common mold species are given in Table 2 on the following page.

Because different mold species grow under different humidity and temperature conditions, and colonize surfaces at different rates, the range of mold species present may also be a direct indicator of the past duration of moisture and humidity conditions, or an indication of continuing sub-surface moisture intrusion.

Table 2. Common Mold Growth Locations in Water Saturated Building Materials

Species Present	Duration of sustained Saturation occurrence	Common Moisture	Surfaces Colonized
1). Penicillium	Single-multiple occurrences	Days	Drywall, boxes, ceiling tiles
Aspergillus sp. Cladosporium	Single-multiple occurrences	Days	Carpeting, baseboards
2). Alternaria,	Multiple occurrences	Days -weeks	Back of ceiling tiles, behind baseboards
3). Stachybotrys Chaetomium Arthrinium	Chronic saturation	Week - months	Back of ceiling tiles, wall cavities
4). Dry & wet rot	Chronic saturation stud members, crawl spaces, flooring	Months - years	Wall cavities on wood

3.1.3 Interpretation of Algal Spore / Fern Spore Category:

When algal spores are detected in any concentration in "indoor" samples, a stagnating water source is likely present nearby. Although significant information is not readily available regarding health effects, algal spores are generally an indicator of persistent moisture and potential bacteriological or protozoa growth.

3.1.4 Interpretation of Pollen Concentrations:

In a typical HVAC supplied air building, surface pollen concentrations will be very low (less than 1 ct/mm^2) or not detected at all and are not a common cause of indoor air quality problems. However, elevated surface concentrations can become entrained by building activity or brought in contact with the respiratory tract through indirect dermal contact. General guidelines are given in Table 3 on the last page.

3.1.5 Interpretation of Surface Skin Cell Fragment (Dander) Concentrations

One of the biggest differences between inside and outside dust concentrations is the predominance of skin cell fragments in the indoor environment. Skin fragments often comprise over 80% of the volume (and mass) of identifiable particles in indoor air as observed by optical microscopy. Although no direct health effects can be diagnosed by their measurement, skin cell fragment concentrations on surfaces are an indicator of occupant density, commensal bacteria potential, general janitorial effectiveness, and HVAC filtration conditions in the building. General guidelines are given in Table 3 on the last page.

3.1.6 Significance of Surface Cellulose / Synthetic Fibers

Airborne fibers including cellulose and common synthetic fibers such as rayon, nylon, lycra, etc can often act as general dermal or nasal irritant sources. Fiber sources include architectural finishes, paper products, clothing, and carpeting, and are commonly found in "clean" buildings. Cellulosic or synthetic carpet or clothing fibers by themselves may not be a direct cause of nasal, eye, or bronchial symptoms, however, elevated surface concentrations of fibers may be an indication of inadequate housekeeping or ventilation, shedding architectural finishes, or high occupancy rates. Surface concentration guidelines are given in Table 3 on the last page.

3.1.7 Significance of Surface Fiberglass Fibers

Airborne and surface fiberglass fibers in the indoor environment are the direct result of the shedding and/or degradation of building materials including but not limited to ceiling tiles, thermal wall and ceiling insulation; external HVAC duct wrap insulation, and internal sound damping insulation utilized in HVAC system mixing boxes and ducting. Occasionally, regulated hazardous fibers such as asbestos may be encountered. These types of fibers pose long term exposure risks, but are not responsible for immediate health related complaints beyond irritant reactions of mucous membranes and skin. Fiberglass fibers can be responsible for skin and mucous membrane irritation and respiratory discomfort at levels below the Permissible Exposure Limit (PEL) of 1 fiber/cc. The scientific literature MEDITEXT (R) database[5] indicates that fibers with diameters greater than 5.0um can produce irritant pruritic dermatitis skin irritation and in rare cases allergic responses involving the upper respiratory tract. Not all fibrous glass fibers will cause equal irritation due to size and morphological differences. Mineral wool fibers found in ceiling tile panels are usually very large, non-uniform in diameter, and possess rounded ends. This type of fiber is less likely to cause irritation and discomfort than sharp-ended fibers. Sharp-ended fiberglass fibers including yellow and pink thermal fiberglass insulation, and black impregnated fiberglass in sound liner (sound dampening) insulation found within the HVAC systems, are likely to cause the greatest degree of irritation. Elevated surface concentrations are an indicator of historical fiber shedding and/or insufficient housekeeping practices. Because fiberglass is relatively large and settles quickly, surface detection can be an indicator of active fiber generation, if the interval between surface cleaning is known, and taken into account. Detection of moderate surface concentrations usually means the potential for skin or eye irritation through indirect contact with contaminated horizontal surfaces exists. Although no standards are available to determine what airborne or surface levels may cause symptoms, a relationship between complaints and high surface measurements is commonly observed. Surface fiberglass concentrations above 10 fibers/mm^2 usually occur only in proximity to actively disturbed sources, and can sometimes be associated with irritation complaints.

Surface concentration guidelines are given on the last page.

3.1.8 Interpretation of Insect Part Concentrations:

The insect part category reports all body parts including antennae, legs, scales, and wing fragments. Differentiation of the type of inspect parts (wings, legs, scales, etc.) is not performed. In "clean" indoor environments, insect parts are not readily encountered in surface samples in quantities exceeding approximately 5.0 cts/mm^2. When encountered, they are usually an indicator of inadequate building maintenance or housekeeping. Occasionally dust mites are found in surface samples when inadequate housekeeping or extensive mold growth is present. The presence of dust mites in surfaces samples may indicative a severe bioaerosol contamination problem.

3.1.9 Significance of Optically “Opaque” Surface Particle Concentrations:

The opaque particle category encompasses a wide range of microscopically similar, but unrelated optically opaque particles of biogenic and inorganic origin. Included in this category are the following sub-groupings of particles:

- Soil & plant particles - mineral particles and decayed plant debris
- Soot particles – Indoor & outdoor fires, fuel combustion, fly ash, tire rubber particles
- Building generated particles - pigment, copier toner, motors & belt particles
- Occupant, insect, & microbiological generated particles - decayed dander, insect droppings
- Rust and corrosion particles

Although these particles can often be characterized without chemical or electron microscopy analysis, positive identification without additional information is often not possible.

High surface opaque dust concentrations are usually associated with a combination of inadequate HVAC filtration, system shedding, biological growth in the system ducting or drip pans, or marginal housekeeping. No known health effects can be directly implied by this category without identifying the source and composition of the particles. Elevated levels should be used as a “yellow flag” to clean up the HVAC system and/or improve housekeeping practices. General guidelines are given in Table 3 on the following page.

It is important to remember that particle concentrations on surfaces are determined by gravitational settling over an extended period of time, and the length of time elapsed since the surface was last cleaned. As a result, dust samples are not necessarily representative of the present condition. In other words, a high airborne mold measurement may not be reflected in the surface concentrations measured at the same time. The species or morphological distribution of certain particle categories are also important. For example, a mold spore concentrations of 15 cts/mm^2 consisting of one species may be an indication of “growth”, whereas if the spore concentrations are comprised of a wide range of species, it may only indicate infiltration and settling. The same holds true for opaque particles. If the counts are primarily from rust-like particles, it may indicate a system corrosion problem, whereas if the opaque particles are from various sources (soil, combustion debris, pigments etc.) it may only indicate high infiltration and settling of background dust.

Table 3. Guidelines for Concentration Ranges of Surface Dust

	Counts per square millimeter (cts/mm^2)		
	Normal (low)	Moderate	High
Mold Spores	0.1-10	10-50	>50
Algal or Fern spores	< 0.5	0.5-5.0	>5.0
Pollen	< 1.0	1.0-5.0	>5.0
Skin Cell Fragments	1-10	10-100	>100
Fiberglass fibers	< 0.1	0.1-1.0	>1.0
Cellulosic fibers	< 1.0	1.0-10.0	>10.0
Insect parts	< 0.1	0.1-1.0	>1.0
Opaque particles	1-20	20-100	>100

All concentration ranges for horizontal surfaces are given on the basis that the surface has not been cleaned less than 1 week prior to sample collection. Concentrations can vary significantly depending on their location in the room, the elapsed time between surface cleaning, and the type of cleaning used.

4.0 REFERENCES

1. E. Grant Smith, 1990. Sampling And Identifying Allergenic Pollens And Molds. Blewstone Press, San Antonio, Texas.

2. Moore P.D., Webb J.A., Collinson M.E., 1991. Pollen Analysis. Blackwell Scientific Publications, Cambridge Mass.

3. Kalliokoski, Juutinen. Fungal Growth And Activity On Moist Building Materials. Department of Environmental Sciences, University of Kupoio, Finland. Proceedings of Indoor Air <93, Vol. 4.

4. MEDITEXT (R) (Medical Management) Database X Effects of fiberglass.