Part 5: Diagnostic Sampling Considerations

Thursday August 30
2:45 – 3:30 p.m.

Develop A Sampling Plan

- A sampling plan may be:
  - Broadly Focused – Are the kinds or concentrations of microbes indoors and outdoors similar?
  - Narrowly Focused – Are specific disease-causing agents present in substantial amounts where the person became ill? Like Aspergillus fumigatus

Broadly Focused Study

- Example: Carnelley et al. 1887
  - Are there more airborne microorganisms in homes with poor housekeeping?

Housekeeping Status	Concentration of Microbes per Liter
Clean	18
Dirty	41
Very Dirty	93

Narrow Focus
Looking for Aspergillus Fumigatus

Location Variables

- Location variables in a building that may be important in developing a sampling plan:
  - Damp vs. dry areas
  - Different HVAC system components
  - Pressurization in attic vs. occupied spaces

Location Variables (Cont'd)

- Example – Airborne *Penicillium chrysogenum* (CFU/m^3) in leaky rooms, non-leaky rooms, and in the outdoor air.

Outdoor air	4
Leaky rooms	211
Non-leaky rooms	6

Source: Morey et al., 2003, Intern. Biodeterioration 52, 197

Location Variables (Cont'd)

- Example – Presurization in attic vs. occupied space in Florida home where attic is positively pressurized by leaky air supply ducts relative to lower (occupied) floors:
  - Condensation occurs on cool (air-conditioned) surfaces in wall cavities and soffits leading from attic to lower floors.
  - Sample in attic and on lower floors; measure pressure differentials; Also sample in outdoor air.

Time Variables

- A recent example of how timing of sampling affects sampling results from D. W. Li, Proc. Indoor Air Beijing, 2005, pp. 1450-1454.
  - The outdoor concentration of boletes basidiospores varied diurnally by 1,000%.
  - Indoor concentrations followed the same diurnal pattern; low of 500/m^3 in morning; high of 4,000/m^3 in evening.
  - So timing of indoor and outdoor comparative sampling is important; also, openness of windows is important.

Comparative Sampling

- Comparative outdoor and indoor sampling for microbial agents can provide a basis for deciding if unusual types of molds occur indoors. Possible occurrence of chronic dampness.
    - Rank order comparison of indoor and outdoor mold spores is important; however, data interpretation may not be straightforward.
    - Remember that physical inspection for visible mold growth is most important.

Comparative Sampling (Cont'd)

This kind of sampling data together with a thorough inspection of the building showing an absence of significant visible mold growth indicates a normal mycological condition:

Fungi in outdoor air (ranked taxa; % of total)
Cladosporium cladosporioides (71)
Epicoccum nigrum (14)
Yeasts (5)
Non-sporulating fungi (4)
Penicillium brevicompactum (2)

Fungi in indoor air (ranked taxa, % of total)
Cladosporium cladosporioides (56)
Non-sporulating fungi (16)
Epicoccum nigrum (10)
Ulocladium chartarum (8)
Yeasts (3)

Morey (2001) in Microorganisms in Home and Indoor Work Environments, Ed. by B. Flannigan et al., pp 275-284

Comparative Sampling (Cont'd.)

Air Sampling for Culturable Fungal Species in a Building with Mold Contamination Problem

Rank order (%)[a] of Fungi Types in	
Outdoor Air	Indoor Air
Cladosporium cladosporioides (92)	*Aspergillus versicolor* (24)
Penicillium brevicompactum (2)	*Penicillium corylophilum* (21)
Cladosporium sphaerospermum (1)	*Cladosporium cladosporioides* (14)
Penicillium implicatum (1)	*Penicillium citrinum* (8)
Penicillium sclerotiorum (1)	*Wallemia sebi* (7)

"Ranked types of Fungi in percent of total."
Source: Adapted from Morey, 2007

Comparative Sampling (Cont'd)

- This kind of sampling data together with an inspection showing substantial hidden mold in room walls is indicative of an atypical mycological condition:

	Concentration	
Mold Taxa	Outdoors	Indoors
C. cladosporioides	207	71
P. chrysogenum	4	211

Source: Morey et al., 2003

Sampling Considerations

- Considerations when carrying out indoor/outdoor sampling:
  - Collect outdoor samples while facing into the wind on the roof or at HVAC outdoor air inlets; or sample away from house in a quiet location.
  - Avoid sampling on a porch or immediately downwind from a building.
  - Avoid sampling outdoors near strong bioaerosol sources (e.g., soil excavation).

The Sampling Method

- The microbial agent of interest often determines the choice of sampling method.
- Culture-based methods are important in identifying microbes to species or subspecies especially where infection is involved (*L. pneumophila* Serogroup #1, *Escherichi coli* 0157/H7, *Aspergillus flavus*).
- Other techniques such as PCR can identify microbes to species or subspecies, but cannot distinguish if microbe is living and is capable of causing infection.

The Sampling Method (Cont'd)

- The collection of airborne allergens, toxins, glucans, and endotoxins is not constrained by loss of culturability. Air sampling can be accomplished by filtration (large volumes of air usually required).
- When collecting mold spores by filtration, it is important to note that some spores (e.g., *Penicillium, Aspergillus, Eurotium* species) have a longer half life or are more desiccation-resistant than other spores (e.g., *Cladosporium, Epicoccum, Stachybotrys* species). See AIHA Field Guide, 2005, p. 99.

Dust Samples

- Settled dust is heterogeneous and can vary in composition to include fibers, soil particles, and microbes in soil, pollen, allergens including those from mites, pets, fungi, and bacteria.
- Dusts may be collected by bags that fit into nozzles of vacuum cleaners, by filter cassette mini-vacuums, or by obtaining the collective dust content of an entire precleaned vacuum cleaner or by using an insert in vacuum nozzle.

Dust Samples (Cont'd)

- In terms of sampling strategy, pre-determine if dust samples should be collected from above floor surfaces (Pinard et al., 2005, Saratoga Springs Conf., p. 191) or from floor surfaces.
- Pre-determine if dust samples are to be analyzed (for fungi) by direct plating (more ecological important species detected) or dilution plating (may over-emphasize species with longest half lives).
  See AIHA Field Guide, 2005, pp. 120 and 58.

DUST SAMPLING

Dust Samples

Allergens in carpet dust samples:

	Allergens (µg/g)	
Source of Dust	DER F1	FEL D1
Complaint Office	0.5	75
Control Office	0.4	5
HVAC Soot	ND	ND

Morey, 2007, Chapter 3; Compliant office was a call center open 24-7; No dust removal by vacuum cleaning.

Dust Samples

- *Stachybotrys* in carpet dust assessed by different types of lab analysis:

	Stachybotrys	*% Stachy*
Analysis	Concentration	Among Taxa
Dilution on MEA	1×10^3 cfu/g	5%
PCR (24 Target)	1×10^7 spore equivalents/g	> 95%

Morey, 2007, Chapter 3.

Cellotape Sampling

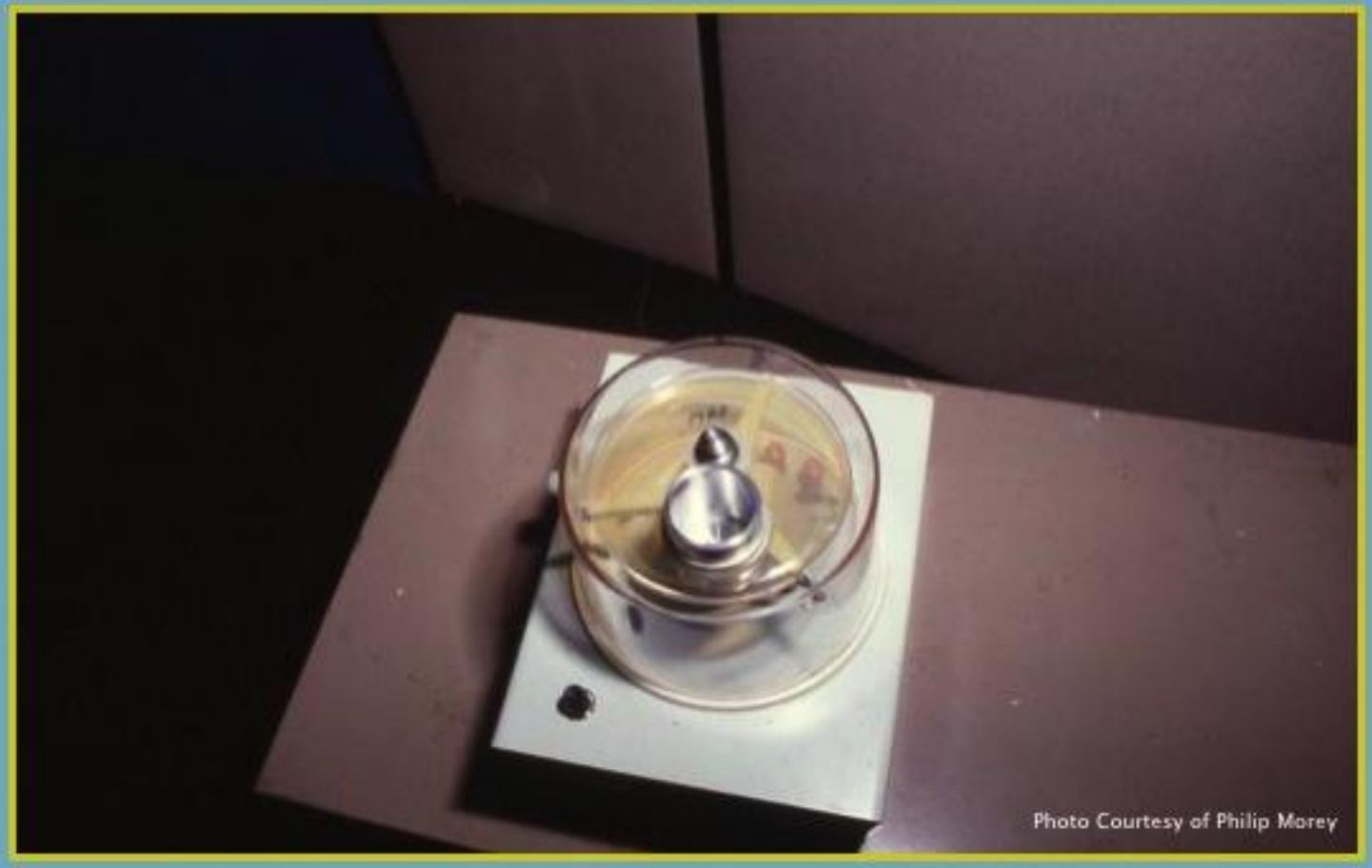
- Cellotape sampling and direct microscopy analysis:
  - Identify the general types of molds that may be present on a surface; carry a microscope.
  - Document whether mold propagules on a surface are characterized by "normal deposition" or as "growth," past or present.
  - "Growth" is the presence of a network of hyphae or fungal sporulating structures.

Tapelift Sample: Mycelium is Present

Cellotape Sampling (Cont'd.)

- Cellotape sampling and direct microscopy analysis:
  - Limitation: Cannot be used for speciation of molds.
  - Limitation: Cannot readily be used to determine if mold growth occurs within or beneath a porous surface (e.g., growth in carpet).

Air Sampling for Culturable Molds; SLIT-TO-AGAR IMPACTOR*



* Useful to see if airborne molds vary in type and concentration over time.

Filtration Air Sampling

- Example of filtration air sampling for endotoxin upwind and downwind from waste water treatment plant:

Sample Location	Endotoxin Units/m^3
50 M Upwind of Plant	14
50 M Downwind of Plant at Entrance to Nearby Office Bldg	11
Above Digester in Plant	58

From Morey 2007, Chapter 3; Why was this sampling carried out?