Limitations of Bioaerosol Sampling in Residential, Work, and Healthcare Environments

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March 2, 2020

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Acknowledgments

Key former and current members of the Hudson COPH Bioaerosols Research Team whose work contributed to this presentation:

- LTC Casey W. Cooper, PhD, Assistant Professor, U.S. Air Force Institute of Technology (AFIT), Wright-Patterson AFB, OH
- Kathleen “Kae” N. Aithinne, Doctoral Candidate and Hudson Scholar, Hudson College of Public Health
- Bradley S. Stevenson, PhD, Associate Professor of Microbiology, Department of Microbiology and Plant Biology, University of Oklahoma

Learning Objectives

- Identify the bioaerosol types of primary concern in occupational safety and health practice
- Recognize the sampling techniques commonly used to assess exposures to these bioaerosols
- Describe the decision tools used to interpret sampling data
- Discuss the limitations of bioaerosol sampling and analysis techniques

Introduction

- Bioaerosols are ubiquitous in the environment and can cause adverse health effects ranging from irritation to fatal infectious disease

- Characterizing bioaerosol exposures is more complex than typical aerosol measurement because we often must culture the captured organism in order to identify and quantify it, and that can be tricky

- In the following, some but not all of the methods available for bioaerosol sampling and evaluation will be discussed, with particular attention to their limitations

Bioaerosol Terminology

- **Bioaerosols** are just airborne particles of biological origin
  - Pollen grains
  - Arthropod fragments (dust mites, cockroaches)
  - Plant proteins (e.g. latex)
  - Animal dander, saliva, urine
  - Fragments of bird droppings
  - Microbes (bacteria, protozoa, fungi, & their byproducts)

Microbial Bioaerosol Hazards

- Infection
  - TB (especially multi-drug resistant TB)
  - *Legionnella pneumophila* (Legionnellosis)
  - *Aspergillus* mold species (Aspergillosis)
- Immune Response
  - Atopic allergy (molds)
  - Hypersensitivity pneumonitis, or HP (allergic extrinsic alveolitis)
- Toxicity
  - Mycotoxins
  - Endotoxins

Size and background concentration

TABLE 19.1 Particle Size and Natural Background Concentration of Bioaerosols[a]

Type of Bioaerosol	Size (μm)	Concentration (number/m^3)
Viruses	0.02–0.3	—
Bacteria	0.3–10	0.5–1000
Fungal Spores	0.5–30	0–10,000
Pollen	10–100	0–1000

[a] Jacobson, AR and SC Morris, "The Primary Air Pollutants – Viable Particles, Their Occurrence, Sources, and Effects", in Stern AC (Ed.), *Air Pollution*, 3rd ed., Academic Press, New York, 1976

Table taken from Hinds WC, *Aerosol Technology – Properties, Behavior, and Measurement of Airborne Particles*, 2nd ed., John Wiley & Sons, New York, 1999.

Mycobacterium tuberculosis

- Shown Wells et al. (1950s) to be transmissible by the airborne route
- Multidrug-resistant TB (MDR-TB) strains are a major challenge worldwide
- Current air sampling approach is collection on filters with analysis by real-time quantitative polymerase chain reaction (RT-qPCR) - not something most of us will ever do

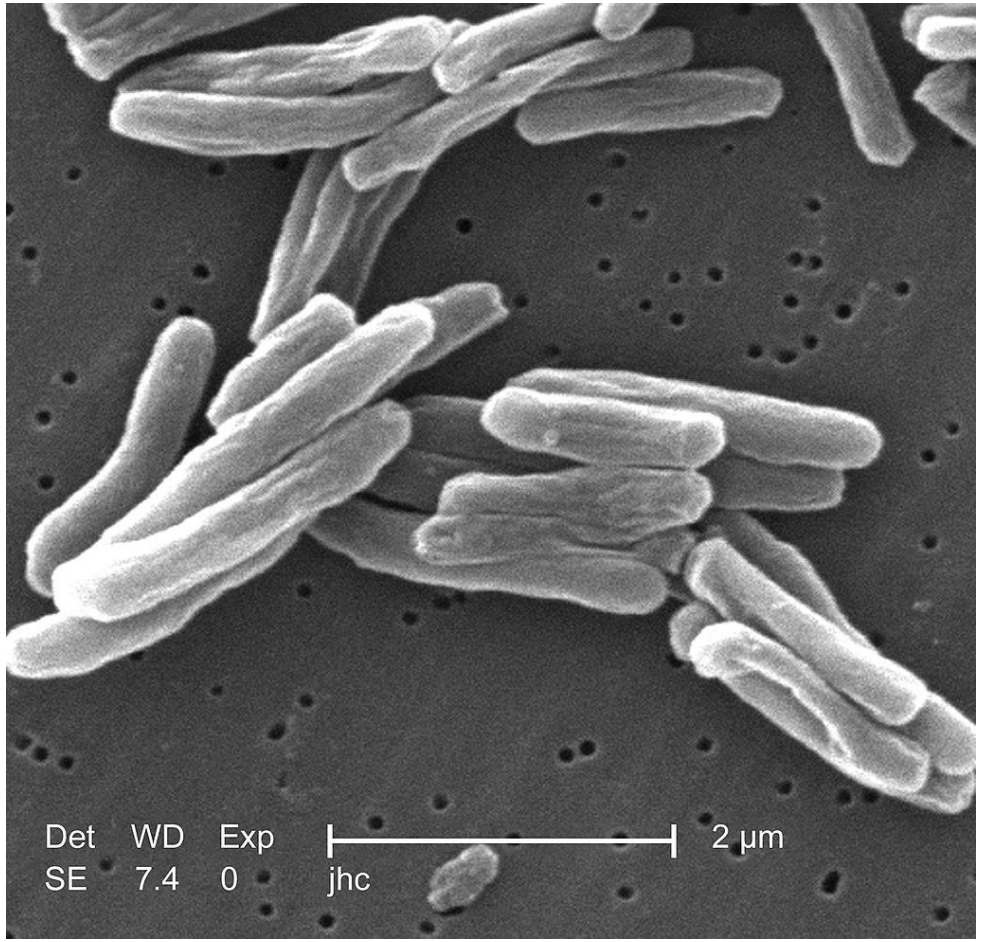


Photo Credit:
https://commons.wikimedia.org/w/index.php?curid=4347472

Norovirus

- "Norwalk-like virus"
- Highly infectious
- Likely transmissible by the airborne route
- Current air sampling approach is impaction on agar plates, extraction, and RT-qPCR analysis – also not something most of us will ever do

Photo credit: https://twitter.com/cdphe/status/433630396257800192

Photo credit: WPTV News, West Palm Beach, FL

Pollens

- Ubiquitous in the environment
- Sampling and quantification is crude, using a Rotorod sampler and manual counting via microscopy – levels are classified e.g. as “low”, “moderate”, “high”
- Regularly monitored by others due to allergy and reported as a weather item

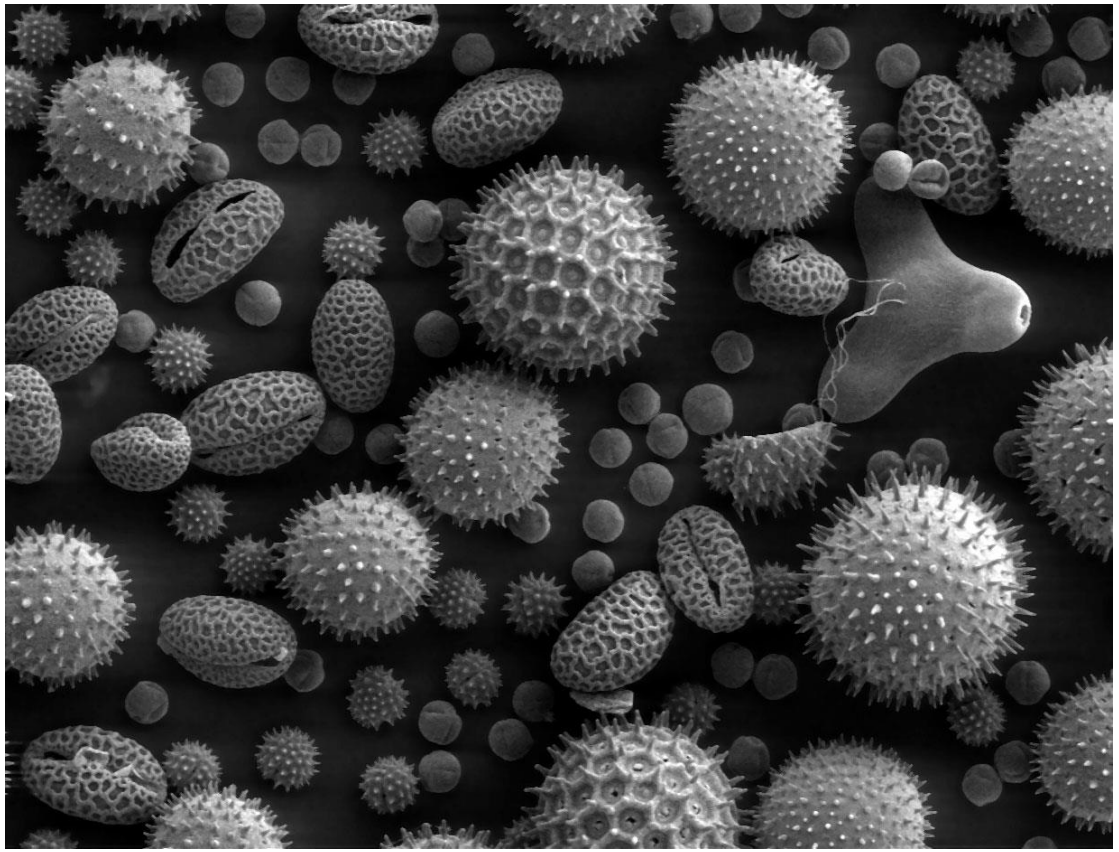


Photo credit: https://commons.wikimedia.org/wiki/File:Misc_pollen.jpg

Rotorod pollen sampler

Spins at 2400 rpm

Rods' leading edges are coated with silicon grease

Grains identified and counted by microscopy after staining

Sampled volume is determined by the rods' projected areas and rotation rate

Results are expressed in pollen grains/m^3

What types of bioaerosol sampling are industrial hygienists likely to do?

Mold spores

Airborne pathogens in healthcare environments

Molds

- There is tremendous public concern about mold health effects
- Particular concern about *Stachybotrys chartarum*, so-called “toxic black mold”, though no association between this mold and particular health symptoms has been proven*
  - Googling “toxic mold” and “toxic black mold” yielded over 800,000 results on 2/4/20

* “Facts about *Stachybotrys chartarum*”, CDC, Dec 2019, https://www.cdc.gov/mold/stachy.htm

Why molds in general and *Stachybotrys* in particular?

- Some molds are known to produce mycotoxins (e.g. *Stachybotrys*)
- Quick-and-dirty epidemiology initially showed an association between its presence in water-damaged Cleveland homes and infant deaths due to acute idiopathic pulmonary hemosiderosis (lung hemorrhage) in 1993
- Tort liability suit decisions have supported this belief – e.g. $32 million Farmers Insurance case in Texas for damage plus mental anguish
- Attorneys, product and service providers, and the press have stoked the public concern
- The science was slow to catch up, so now mold toxicity it is generally accepted as fact

The Science: CDC and *Stachybotrys*

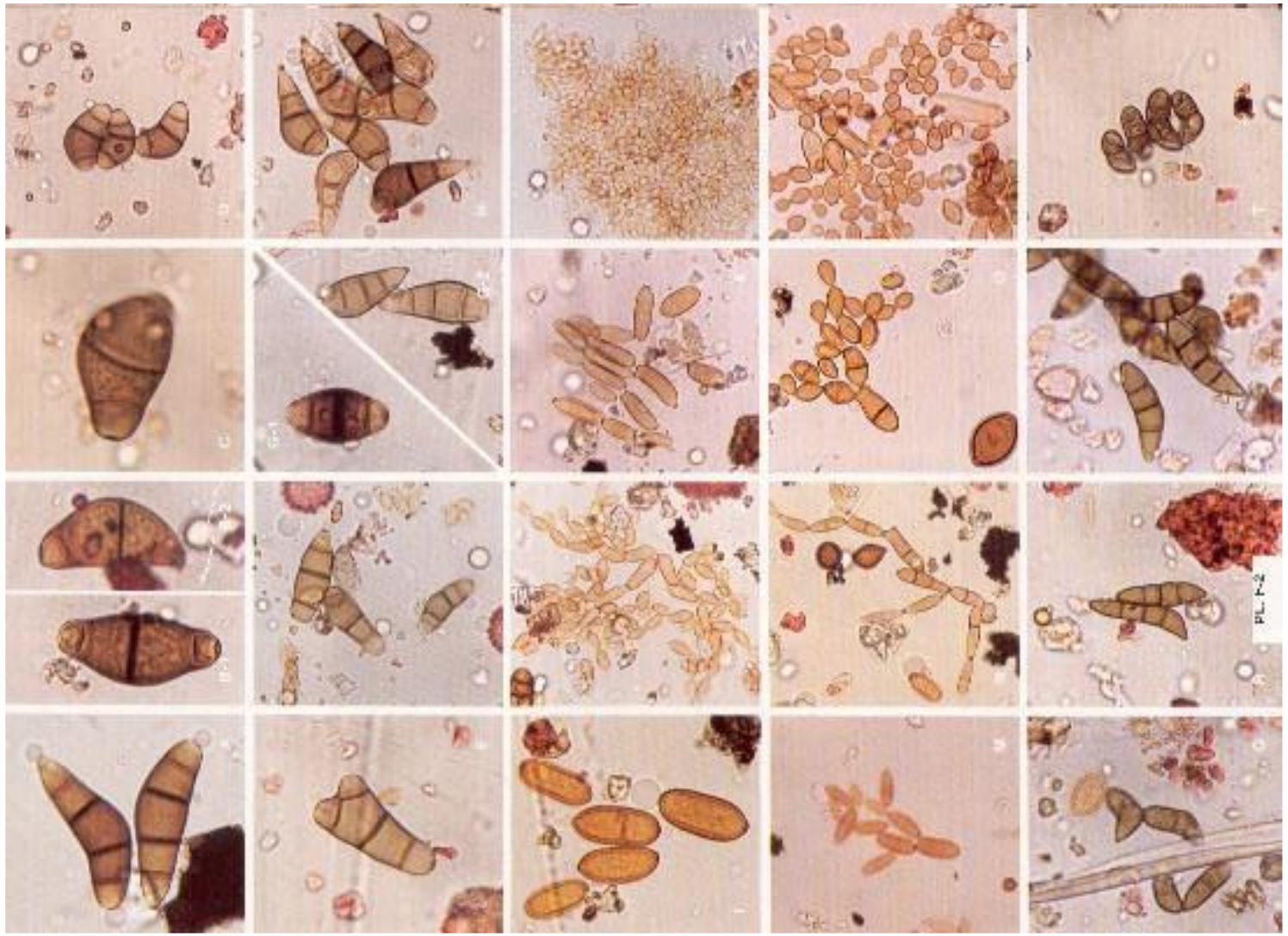
"Both groups of reviewers [internal CDC and external scientists] concluded that the available evidence does not substantiate the reported epidemiologic associations … between household water damage and AIPH [acute idiopathic pulmonary hemosiderosis] or between household fungi and AIPH … or any inferences regarding causality."

MMWR 49(09):180-4, March 10, 2000, "Update: Pulmonary Hemorrhage/Hemosiderosis Among Infants --- Cleveland, Ohio, 1993-1996"

"The term "toxic mold" is not accurate. While certain molds are toxigenic, meaning they can produce toxins (specifically mycotoxins), the molds themselves are not toxic, or poisonous. **Hazards presented by molds that may produce mycotoxins should be considered the same as other common molds which can grow in your house.** There is always a little mold everywhere – in the air and on many surfaces. There are very few reports that toxigenic molds found inside homes can cause unique or rare health conditions such as pulmonary hemorrhage or memory loss. These case reports are rare, and **a causal link between the presence of the toxigenic mold and these conditions has not been proven."**

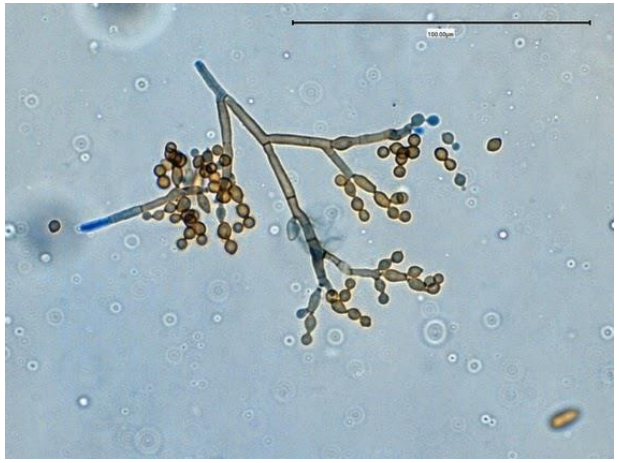
https://www.cdc.gov/mold/stachy.htm

- There are thousands of mold types
- Mold spores are everywhere - outdoors and indoors
- Many spores, but not all, have distinctive shapes and can be identified by optical microscopy



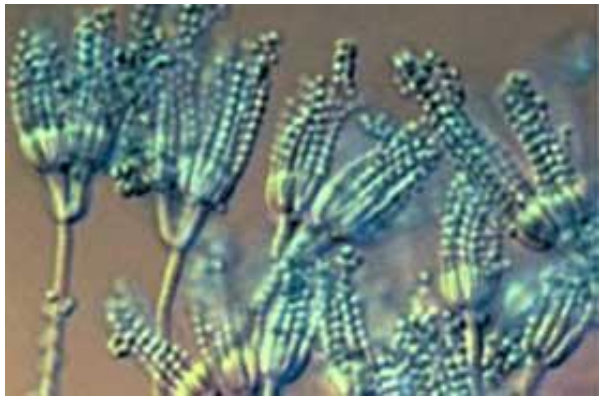
Example page from Smith, E.G, Sampling and Identifying Allergenic Pollens and Molds, Blewstone Press, San Antonio, 2000

Some of the most common mold types



Cladosporium

Alternaria



Penicillium

Aspergillus

Typical Outdoor Mold Spore Concentration Ranges

Description	Spores (cts/m³)	Predominant Types *
Arid / desert regions	50 – 5,000	Cladosporium, asco/basidospores Alternaria, Penicillium, Aspergillus
Urban & coastal strip	200 - 10,000	Cladosporium, asco/basidospores Alternaria, Penicillium, Aspergillus
Inland valley & native vegetation	500 - 20,000	Cladosporium, asco/basidiospores Penicillium, Aspergillus
Farms & heavy forestation	5,000 - 50,000	Cladosporium,asco/basidiospores Alternaria, Penicillium, Aspergillus

•Genus/category listed in order of decreasing concentration frequency

Typical Indoor Mold Spore Concentration Ranges

Description	Spores (cts/m³)	Predominant Types *
"Clean" non-HVAC supplied Buildings.	less than 2,000	Total for all spore types
"Clean" HVAC supplied buildings	less than 1,000	Total for all spore types
Both types of buildings		less than 700 Penicillium, Aspergillus
Possible Indoor Amplification	1,000 - 5,000	Penicillium, Aspergillus, Cladosporium
Indoor Amplification likely present	5,000 - 10,000	Penicillium, Aspergillus, Cladosporium
Chronic Indoor Amplification	10,000 - 500,000	Penicillium, Aspergillus, Cladosporium
Inadequate flood cleanup or active Indoor demolition of contaminated surfaces	50,000 - 10,000,000	Penicillium, Aspergillus, Stachybotrys, Cladosporium, Chaetomium, Basidiospores Tricoderma, Ulocladium, etc.

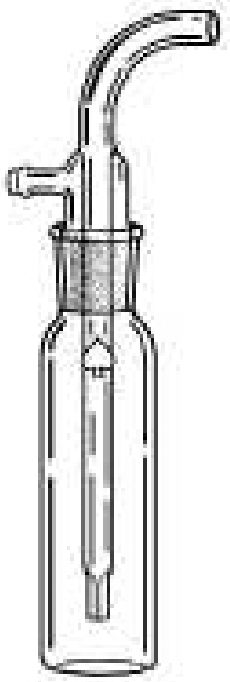
From: Environmental Analysis Associates Indoor Air Quality Laboratory, *Air-O-Cell Method Guide & Particle Atlas*, San Diego, CA, http://eaabaxter.com/docs/air-o-cell-methodguide-atlas-2013.pdf

Mold sampling methods

- Viable or “culture”
  - Microbes are deposited directly on culture media or captured and plated onto media
  - Plates are incubated and the colonies identified and counted by optical microscopy
  - Results are expressed in Colony Forming Units per cubic meter (CFU/m^3)
- Non-viable or “spore trap”
  - Spores are deposited on a surface, or captured in a liquid, and transferred to a microscope slide for identification and counting via optical microscopy
  - Results are expressed in fungal structures per cubic meter (fs/m^3)

Culture-based mold sampling with impingers

- Liquid impingers with culture plating
  - AGI-30 Impinger
  - SKC BioSampler
  - Both sample at 12.5 L/min with 20 mL collection liquid
  - Plates are enumerated in CFU/m^3



AGI-30 Impinger

SKC BioSampler

Culture-based mold sampling directly onto culture plates

- Multi-hole jet-to-agar impactors
  - Andersen 6-stage cascade jet-to-agar impactor (research only) – 28 Lpm
  - Andersen N-6 single-stage jet-to-agar impactor (most commonly used in the US) – 28 Lpm
  - MicroBio (commonly used in the UK) – 100 Lpm

Andersen 6-stage

MicroBio Sampler

N6

N6 plate – 400 jets

Positive hole correction for jet-to-plate impactors

- Multiple microbes can be deposited at a given jet's location in a multi-jet impactor (e.g. N-6, MicroBio)
- Correction can be applied to account these coincidence or "positive hole" errors that would otherwise cause an underestimation of the concentration

TABLE 19.2 Correction Factors for Multiple Particle Collection in Multijet Impactors[a]

Filled Fraction[b]	Correction Fraction[c]	Filled Fraction	Correction Factor
0.05	1.026	0.55	1.452
0.10	1.054	0.60	1.527
0.15	1.084	0.65	1.615
0.20	1.116	0.70	1.720
0.25	1.151	0.75	1.848
0.30	1.189	0.80	2.012
0.35	1.231	0.85	2.232
0.40	1.277	0.90	2.559
0.45	1.329	0.95	3.154
0.50	1.386	1.00	>5.878

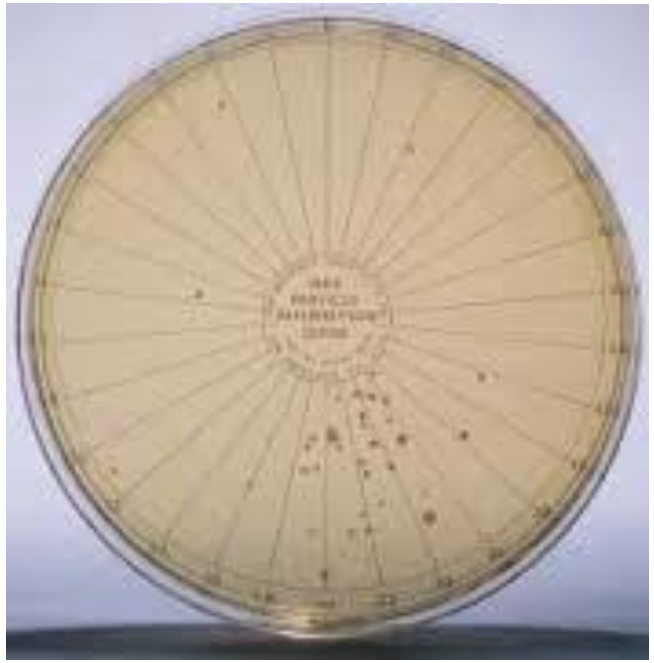
[a]Calculated from Macher (1989).
[b]Fraction of deposition sites with colonies.
[c]Total number of viable particles collected equals the number of filled sites times the correction factor.

Table taken from Hinds WC, Aerosol Technology – Properties, Behavior, and Measurement of Airborne Particles, 2nd ed., John Wiley & Sons, New York, 1999.

Culture-based mold sampling onto plates (cont.)

- Slit-to-agar viable impactors
  - AirTrace (Particle Measuring Systems, Inc.) rotating plate slit-to-agar sampler
  - 150 mm (15 cm) diameter plate
  - Time-resolved concentration information
  - Expensive - research instrument

AirTrace



150-mm plate
with 70 mL agar

Limitations of Viable Sampling

- Culturing takes time (1 to 3 weeks)
- Choice of culture media and incubation conditions influences what grows and how fast it grows in culture
  - Fast-growing colonies may obscure slow-growing colonies
  - Some organisms that are present may not grow at all
- In multi-jet impactors, “coincidence” of deposits may obscure some of the microbes, so concentrations of specific genera may be underestimated (positive hole correction will not address this)
- The sampling technique may damage or kill organisms by mechanical stress, desiccation, oxygen toxicity, etc., or bury them too deeply in the agar to allow growth

An cautionary tale – *Clostridioides difficile*

- *C. difficile* is an endospore-forming gastrointestinal pathogen that can cause severe to fatal diarrhea in hospital patients
- 500,000 infections and 26,000 deaths per year
- Reliance on “contact precautions” to prevent hospital spread is only moderately successful
- Endospores likely are spread outside patient rooms on air currents, like TB
- However, air sampling using standard liquid impinger methods has been unsuccessful in detecting airborne *C. difficile* spores in hospitals, and jet- and slit-to-agar impactors have rarely detected them

Our studies with *C. difficile*

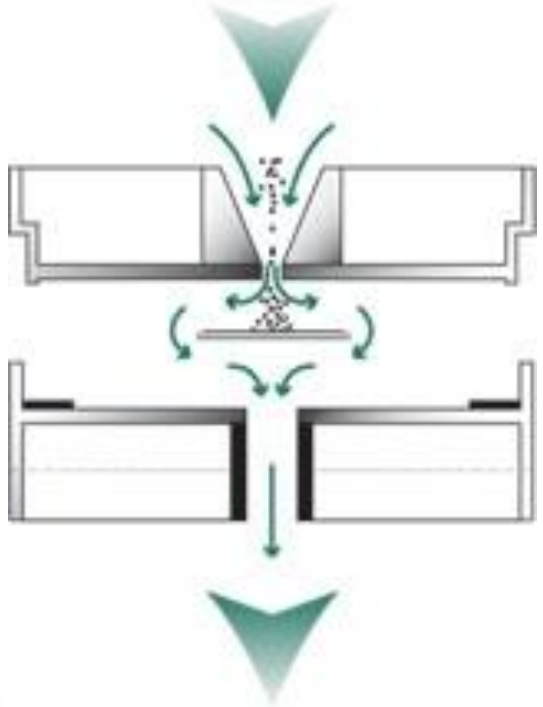
- *C. difficile* spore bioaerosols are generated in high numbers during toilet flushing, and remain airborne for extended periods[1]
- The "gold standard" slit-to-agar sampler (AirTrace) substantially underestimates the airborne concentration[2]
- Standard liquid impinger techniques do not work with *C. difficile* (the captured spores are rendered non-culturable)
- Fortunately, we have developed new sampling methods that appear to work quite well (manuscripts in review or preparation)

[1] Aithinne, K., C. Cooper, R.A. Lynch, and D.L. Johnson (2019). Toilet plume aerosol generation rate and environmental contamination following bowl water inoculation with *Clostridium difficile* spores. American Journal of Infection Control 47(5):515-520.

[2] Cooper, C., K. Aithinne, E. Floyd, B. Stevenson, and D. Johnson (2019). A Comparison of Air Sampling Methods for *Clostridium difficile* Spore Aerosol. Aerobiologia, published online February 8, 2019, https://link.springer.com/article/10.1007/s10453-019-09566-2.

Non-viable methods

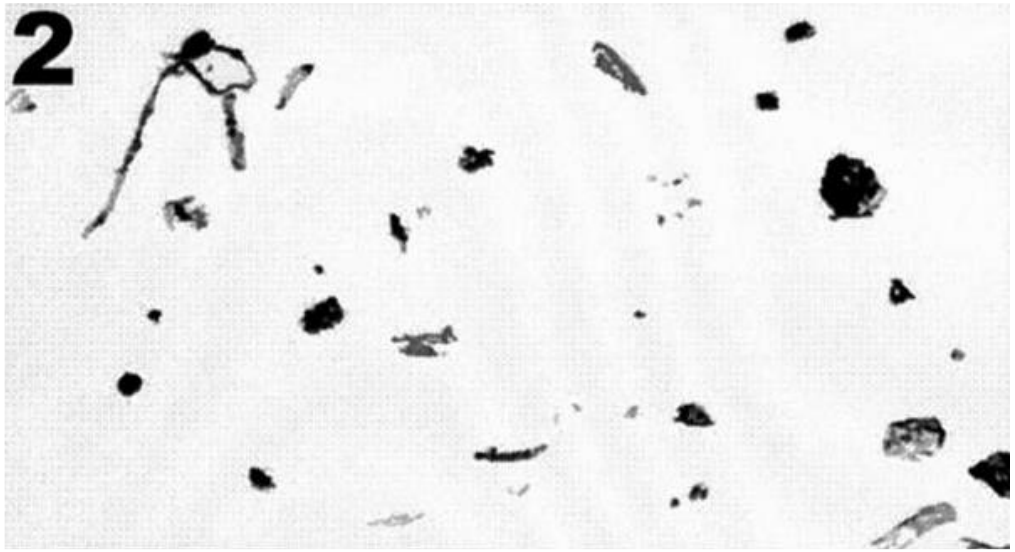
- Liquid impingers with microscopy
  - AGI-30 and SKC BioSampler are the most commonly used
  - Spores are collected by the impinger liquid and particles are counted by microscopy
- Impactor with microscopy
  - Air-O-Cell is the most commonly used
  - Spores trapped on a sticky surface are counted by microscopy



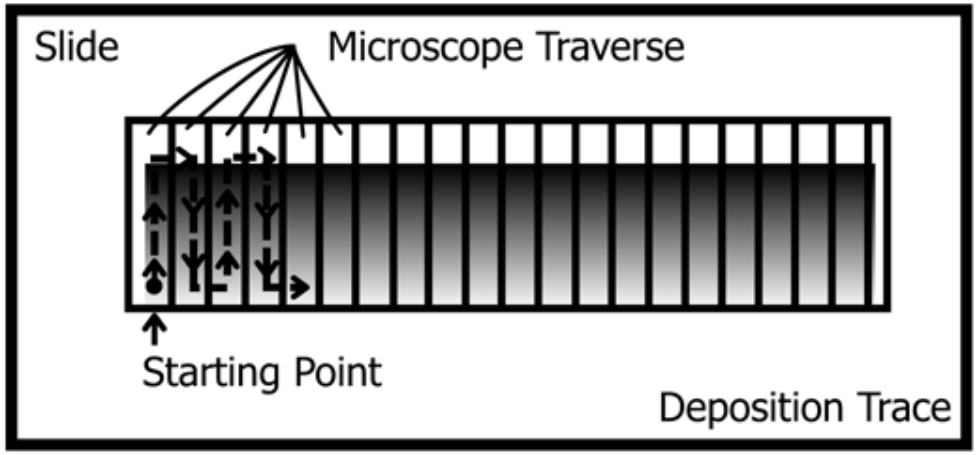
Images: Zefon.com

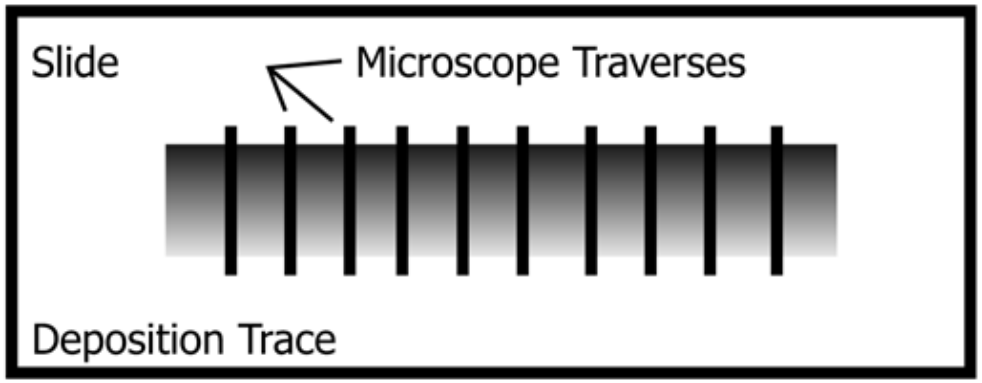
Air-O-Cell Counting

- ASTM D7391 - 17e1: Standard Test Method for Categorization and Quantification of Airborne Fungal Structures in an Inertial Impaction Sample by Optical Microscopy (2017)



ASTM debris rating 2 - approximately 5 % to 25 % of the trace occluded with particulate matter.





Limitations of Non-Viable Sampling

- Optical microscopic identification and counting requires quality microscopes and highly trained and experienced analysts
- Even with highly trained and experience analysts using quality equipment, counting variability is very high (coefficients of variation (CV) averaged approximately 100% in a 2011 ASTM inter-laboratory study)
- Results are subject to both positive (over-counting) and negative (under-counting) bias
- Both living and dead organisms are counted, so viable concentrations are likely to be overestimated

ASTM D7391 – 17e1

"It must be emphasized that the detector in this test method is the analyst, and therefore results are subjective, depending on the experience, training, qualification, and mental and optical fatigue of the analyst."

A major limitation of both viable and non-viable measurements

- Even if we had accurate measurement data, there are no exposure standards!

"The lack of health-based exposure criteria for most types of biological agents precludes environmental sampling and simple comparison of measurements with established air concentrations and dose-response relationships."

(Bioaerosols Assessment and Control, ACGIH Bioaerosols Committee, 1999)

The bottom line question with mold: Is there an indoor source that we cannot see?

If there is an obvious indoor source

- Identify and eliminate the moisture source
- Remediate the mold in accordance with current guidance, e.g.:
  - “Guidelines on Assessment and Remediation of Fungi in Indoor Environments”, New York City Department of Health & Mental Hygiene, Bureau of Environmental & Occupational Disease Epidemiology

NYCDHMH Remediation Levels

- Level I
  - Small isolated areas (< 10 ft^2) – maintenance staff, gloves, goggles, N95 respirator, no containment
- Level II
  - Mid-size isolated areas (10-30 ft^2) – maintenance staff, gloves, goggles, N95 respirator, some containment
- Level III
  - Large isolated areas (30-100 ft^2) – hazmat training, gloves, goggles, N95 respirator, some containment
- Level IV
  - Extensive contamination (> 100 ft^2 contiguous) – hazmat training, full-face HEPA respirator, disposable clothing, negative pressure containment
- Level V
  - Remediation of HVAC systems
    - Areas < 10 ft^2
    - Areas > 10 ft^2

But …

- What if there is no clear indication of indoor mold growth?
  - Little or no visible mold growth
  - No odors typical of molds
  - Non-specific symptoms in some occupants

- Maybe a hidden source?
  - Inside walls
  - Behind wallpaper
  - In attics or crawlspaces

- Is quantitative air sampling useful in deciding if there is an indoor source?

Typical Scenario

An office manager believes the building may be contaminated with mold, but no visible mold growth is present. An Industrial Hygienist is called in to perform a mold evaluation, and chooses to conduct N6 viable sampling for airborne mold spores.

Scenario - sampling

The IH collects 3-minute samples at multiple locations inside the business as well as outdoors at an air entry point.

Scenario – the Data

	Alternaria	*Aspergillus*	*Cladosporium*	*Mucor*	*Penicillium*	*Rhizopus*	*Verticillin*
Outside 1		39	81	10	3	6	
Outside 2	6	42	69		15		
Conf Rm					2	10	
Comm Rm		32	4	5			
Mech Rm						3	
Rest Rm			14		4		1
Store Rm			2	1			
Office A	7		51		5		
Office B			33		2		
Office C			75			5	
Office D			44	2	18		
Office E	9		54	1			
Office F			90	2			

Is there an indoor mold source, or not?

How to Interpret the Data?

Statistical Techniques

vs.

“Professional Judgment”

Statistical Techniques

- Compare a measured value against an established standard (i.e. the Exposure Limit approach)
  - The problem: There are no established standards
- Compare indoor and outdoor locations to look for differences
  - The problem: Airborne spore are lost as air moves from outdoors to indoors. This precludes comparing indoor and outdoor concentrations directly. Concentrations could be corrected for these losses with an assumed “indoor-outdoor loss factor”, but this is not reliable.

Statistical Techniques (cont.)

- Compare the “relative frequency” of mold types indoors and outdoors - rank the concentrations of the different mold types from highest to lowest, and compare the order of the rankings indoors vs. outdoors rather than the values of the concentrations (Spearman Rank Correlation test)
    - The problems: (1) This would only be valid if it can be assumed that the “indoor-outdoor loss factor” is the same for all mold types and all indoor locations sampled; (2) Assumes no lag time for spores to migrate indoors; (3) Requires 5 or more of the same mold types in both the indoor and outdoor samples; and (4) Tests done using data with very low concentrations may give false positive results

Garbage in, garbage out: How good are the data that would be used in these tests?

- Do the sampling/measurement techniques give a true measure of the types and concentrations present? (Are they accurate?)
- How much variability is there in the measures? (Are they precise?)
- Do they reflect the actual conditions in the indoor space over time? (Are they representative?)

Accurate?

- Spore counts by microscopy
  - Molds cannot always be distinguished from the appearance of their spores, so there may be misclassification in spore counts

- CFU counts by culturing
  - The choice of culture medium and conditions affects which molds will grow and how fast (differential growth / overgrowth)
  - Multiple spores depositing at the same point produce only one CFU (coincidence)

In both techniques, the knowledge and experience of the analyst are critical to accurate identification and counting

Precise?

- Mold sampling is notoriously imprecise
  - Side-by-side samples taken at the same time and same location often produce wildly different results
  - Microscopy counting techniques are highly variable (high CV)
- Poor precision greatly reduces the ability (Power) of statistical tests to identify a difference when one actually exists
  - This can lead to a false conclusion about the presence of an indoor source, either that there IS one or there IS NOT one, depending on which test is used

Representative?

- Airborne spore counts and diversity vary dramatically season-to-season, week-to-week, day-to-day, and even hour-to-hour
- Rain, snow cover, and wind affect outdoor counts
- Indoor activity, soiling, and HVAC operation can affect indoor counts

Airborne samples represent a “snapshot” of concentrations indoors and outdoors on the day and at the time of sampling. Conclusions made from limited data about the possible presence of an indoor mold source may very likely be wrong.

Professional Judgment

If the nature of mold sampling data prevents us from using statistical tests with confidence, can the judgment of knowledgeable and experienced professionals provide a reliable alternative when interpreting sampling data?

Back to the Data

	Alternaria	*Aspergillus*	*Cladosporium*	*Mucor*	*Penicillium*	*Rhizopus*	*Verticillin*
Outside 1		39	81	10	3	6	
Outside 2	6	42	69		15		
Conf Rm					2	10	
Comm Rm		32	4	5			
Mech Rm						3	
Rest Rm			14		4		1
Store Rm			2	1			
Office A	7		51		5		
Office B			33		2		
Office C			75			5	
Office D			44	2	18		
Office E	9		54	1			
Office F			90	2			

Is there an indoor mold source, or not?

Johnson et al.* Study

- Mold sampling results from 30 cases sent to IAQ professionals – no other information provided (e.g. water history)
  - CIH or CSP
  - Masters or higher degree in a science-related field and working in indoor air quality or industrial hygiene
  - Bachelor's degree in a science-related field and at least 5 years of IAQ or IH experience

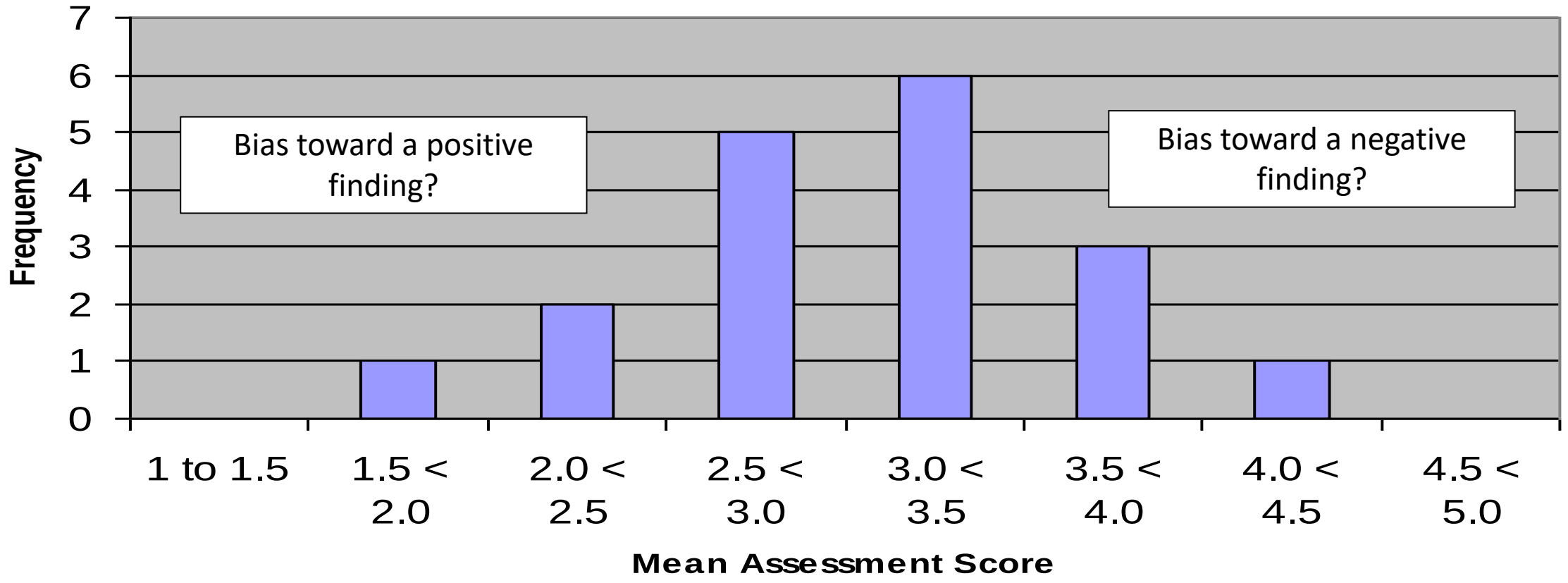
* Johnson, David L., David M. Thompson, Rodney E. Clinkenbeard, and Jason Redus (2008). Professional Judgment and the Interpretation of Viable Mold Air Sampling Data. Journal of Occupational and Environmental Hygiene 5(10):656-663. doi: 10.1080/15459620802310796. PubMed PMID: 18668405.

Johnson et al. Study (cont.)

- Each of the 30 cases was assessed as:

  1 = Definitely mold contamination
  2 = Likely mold contamination
  3 = Not enough information to judge
  4 = Likely no mold contamination
  5 = Definitely no mold contamination

Mean Assessment Scores
18 Reviewers, 30 Mold Sampling Data Sets
(1 = Definitely Yes, 3 = Likely Yes, 3 = Not Enough Info, 4 = Likely No, 5 = Definitely No)



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Agreement between the judges was statistically significantly greater than would be expected by chance, though only slightly (p = .001)

Back to the data again: Case No. 7 from Johnson et al.

	Alternaria	*Aspergillus*	*Cladosporium*	*Mucor*	*Penicillium*	*Rhizopus*	*Verticillin*
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Definitely Yes	Likely Yes	Not Enough Info	Likely No	Definitely No
3	3	7	3	2

Observations on the findings

- The majority of professionals had difficulty drawing a firm conclusion, if any conclusion at all, from the data
- There is only weak agreement between judges -, little better than chance
- There appeared to be consistent differences in the judgments across investigators, indicating individual biases
- 16 of the 30 cases (>50%) received both “Definite Yes” and “Definite No” judgments

What Does this Study Suggest About Professional Judgment?

- Professional judgment is probably NOT a reliable alternative to statistical data analysis
- Therefore, economic decisions based on professional judgment are subject to challenge
- Bottom line: there is a professional liability exposure for industrial hygienists who make professional judgments from mold sampling data

Some conclusions about bioaerosol sampling for mold spores

- Data from airborne mold sampling is likely to be inaccurate, highly imprecise, and non-representative of long-term conditions
- Statistical methods available to analyze mold air sampling data are of very limited use due to the lack of numeric standards and the imprecision in the data
- Professional judgment does not appear to be a reliable alternative to statistical methods

The mold sampling take-away message

- Professional judgments based on limited sampling data may be subject to challenge (including court challenge), with possible professional liability exposure

- An Industrial Hygienist engaging in mold assessment work should have an in-depth understanding of the limitations of mold sampling data and any statistical tests employed in its analysis, and should be extremely careful in rendering professional judgments

Summary

- Bioaerosols are ubiquitous in the environment
- Some bioaerosols are a health concern due to allergenicity, pathogenicity, or toxicity
- Bioaerosol measurement involves another layer of complexity due to viability effects
- While methods exist for bioaerosol sampling, there are no reliable data interpretation tools (standards, statistical methods, or professional judgment)

Limitations of Bioaerosol Sampling in Residential, Work, and Healthcare Environments

Questions?