Strategies for Mold Investigations and sampling

Participants will be in listen only mode

9 a.m. (PST

Continuing Education Units
(CEUs)

To receive a certificate of attendance you must complete the survey after the webinar:

Click on the survey link in the “Thank you” email (sent 1 hour after this webinar)

Complete survey within 24 hours

You will receive an email in 2-3 weeks when your certificate is ready.

Introduction

No reliable guidelines for interpreting indoor mold data

Let's be as scientific as possible

- **-developing hypotheses**
- **-planning steps for testing hypotheses**
- **-establishing criteria for hypothesis acceptance**

Guidelines

* No widely accepted standards exist for any type of fungal sampling (surface, dust, aerosol).

*In 1986, 1987, and 1989, the ACGIH published numerical guidelines. In 1999, they took them back.

- Scientifically valid numerical guidelines are unlikely to exist in the near future. Each case must be considered individually.
- Sound impossible? Perhaps.
- You will never use sampling data as the only or even the primary information on which to base recommendations

Exposure vs. Dose

*Exposure: is an agent entering the body of a person

*Dose: how much of the agent is internally available to the host.

- Note that mold agents are usually carried on particles (spores)

-The agents are born on or within the spores and are released after deposition

-The amount of agent on each spore is unknown

-Thus, you cannot document dose of any mold disease agent

-without dose, you cannot document cause-effect relationships.

Field/Lab Notebooks: Importance and content

- Write everything down!!!
- Use a bound notebook or a digital device backed up frequently
- Write legibly !!!
- Name notebooks/files with unique name and start date
- Entries should be by time and date (sequential)
- For case-specific notebooks
  - - In main notebook write date and time
  - -In main notebook writ4 date and time
  - State that further entries for the case will be in a new not6ebook or file titled: e.g., David Jones Residence, March 12, 2009

Fungal Data Interpretation

- Unless you are an M.D., be aware that interpreting fungal data with respect to health effects can get you into legal difficulties
- *The same is true if you are an M.D. but are not trained in aerobiology
- * With a great deal of work and collaboration you may be able to establish an association between exposure and symptoms, but you cannot establish cause and effect

Mold Investigative strategies - overview

1. Develop preliminary hypothesis from initial phone/letter contact
2. Plan and carry out visual inspection
3. Revisit hypotheses and create new ones if necessary
4. Decide if sampling is needed
5. Design sampling strategy
6. Conduct sampling and analysis
7. Interpret data
8. Make recommendations

Hypothesis Development

*Hypotheses that address only the environment

"Is there mold in this crawl space?"

"Are mold spores from the crawl space entering the occupied space?."

*Hypotheses that address human exposure

"Is this group of people being exposed to this agent?"

"Is the mold on this wall leading to human exposure?"

The potential for exposure

- Questions/hypotheses
  - Is the mold in this crawl space entering the breathing zone of the occupants?
  - Is the *Stachybotrys* on this wall being released into the breathing zone of the occupants?
  - Over time, how much *Stachybotrys* reaches the breathing zone of the occupants?

Null Hypotheses

The negative case

- Hypotheses must address the negative case to avoid bias.
- The negative case cannot be unequivocally proven
- Testing a negative hypothesis requires carefully constructed strategies to achieve interpretable outcomes

Hypotheses resulting from visual inspection

Environmental hypotheses

- There are neither odors nor mold
- There are odors but no obvious mold
- There is visible mold in the occupied space

Exposure hypotheses

- There is no ongoing exposure from indoor sources
- Hidden mold is releasing spores and/or VOCs
- Visible mold is releasing spores and/or VOCs

Sampling Strategies - overview

Type of sample

Analytical method

Sites for sampling

Numbers of samples

Volume of each sample

Sample Types, Analytical methods

Surface samples (microscopy, culture)

Bulk samples (microscopy, culture, PCR, chemical)

- Not quantitative. Good for identifying discoloration as mold or the type of mold present
- Air samples (microscopy, culture, PCR, chemical
- Never the primary step for determining the presence/absence of mold GROWTH; can be used to evaluate exposure with a carefully designed protocol

Sample sites

If possible, photograph the exact site from which the sample was collected and label the photo to connect with the sample

Surface samples

- Obvious or suspected mold growth
- Undisturbed settled dust

Bulk samples

- Vacuum dust
- Pieces of material (fabric, construction material)
- Water or other liquids

Sample site (cont'd)

- Air samples
  - Outdoor air
  - Indoor air near entry
  - Indoor air near mold growth
  - Near potential sources before and during aggressive action
  - Indoor control samples

Number of samples

- As many as you can afford
- At least duplicates at each site under each condition
- Base this on your expert opinion about how variable the aerosol is likely to be

Volume of each sample

- Bulk sample
  - 1 gram of dust
  - Piece with representative sit4es for damping by analyst (Mark the part you want sampled)
  - 5ml liquid
- Air sample
  - 75-100 liters with cassette sampler;
- This applies to outdoor and indoor control sample and indoor non-aggressive mold samples
  - Indoor aggressive 75 – 100 liters in increments of 10-20 liters

Analytical Methods – Microscopy

Sample is placed under a microscope at 100-1000x magnification and looked at directly.

- Is it mold?
- Are spores being produced?
- What kind of mold? (species usually not identifiable).
- Many spores cannot b identified even to genus or group

Analytical methods - culture

Bulk samples are washed and dilution cultured

Swab samples are streaked directly or washed and dilution cultured

Air samples on plates are incubated directly

- Is living mold present?
- What kind of culturable mold is present?
- Note that: the dominant mold in the sample may not be the one that grows in culture
- Cultured air samples always underestimate the actual numbher of spores present, and bias the population toward easily cultured fungi.

Polymerase Chain Reaction (PCR)

Sample is processed to break cells and release DNA.

The DNA is cloned then submitted to electrophoresis.

Resulting bands are compared to standards

- Tells you whether or not DNA (or RNA) of specific fungi is present
- Fungi not included in the assay will be ignored
- Does not tell you about viability
- Is marginally quantitative
- There is no database for comparing concentrations

Chemical methods

GCMS (Gas chromatography/mass spectroscopy.)

- Testing for mold-related VOCs
  - Use only when it is the VOCs that interest you. Mold can e present without measureable VOCs

  Testing for specific toxins

  Use on air samples to estimate exposure

  Do not use on fungi recovered in culture. Toxin patterns will not be representative

Immunoassays (ELISA)

Used to detect specific allergens

Available assays

- Cockroach, dust mite
- Rat, mouse
- Dog, cat, rodent
- *Alternaria*
- Requires at least a milligram of dust

Immunoassays

There are immunoassays that have been developed for measurement of some mycotoxins.

These methods have yet to be rigorously tested for specificity and reliability

Case Study for hypothesis Development

You receive a call from a client who complains of moldy odors in a hoe she wishes to buy.

She wants to know what is causing the odors and whether or not they can be corrected or removed.

Possibilities

There is visible mold in the occupied space

There is mold in non-occupied spaces

There is hidden mold

There are no odors

Visual Inspection

- Look for obvious mold and water damage
- Look for conditions that could lead to mold growth
- Look for “invisible mold and water damage
- Look for other source for volatiles that might be responsible for odors

Hypotheses resulting from visual inspection

Exposure hypotheses

- There are neither odors nor mold
- There are odors and hidden mold is present
- There is visible mold that is causing odors

Hypotheses resulting from visual inspection (cont'd)

Exposure hypotheses

- There is no ongoing exposure from indoor sources
- Hidden mold is releasing spores and/or VOCS
- Visible mold is releasing spores and/or VOCs

- Note that these are hypotheses, not answers.
- Strategies will be developed test each of these.

Hypothesis #1 No Odors, no mold

Negative case – decide on risk of no further effort.

Sample in case you missed hidden mold

- **Sample air at electric outlets (microscopy)**
- **Collect tape sampled of undisturbed dust (microscopy)**
- **Collect indoor/outdoor air samples (microscopy)**
  - **Collect same volume of air indoors and out**

Hypothesis #1 - Interpretation

Hidden mold is absent

- No dominant fungal spores in wall samples or in tape dust samples
- Indoor and outdoor fungal populations similar in quality
- Indoor concentrations by spore type lower than those outdoors

Hypothesis #2 – Odors, but no mold

Assume the odors are mold related, and design samping strategy to locate the mold growth (which is not obvious.

Use strategy similar to that for hypothesis #1

Hypothesis #2 - Interpretation

Hidden mold absent – See strategy 1

- **Consider other sources for odors**

- **Hidden mold present**
  - **Penicillium/Aspergillus dominant on tape samples and/or**
  - **A single spore type dominates wall samples and both spores and hyphae are present.**

Hypothesis #3 – Visible Mold

Probably no sampling needed

Sample to prove to occupant/realtor that what you see is mold

- **Surface samples of apparent mold growth (microscopy)**
  - **Tape samples preferred**
  - **Not quantitative**
  - **Preserve microscopic structures for ID.**

Hypothesis #3 – interpretation

Not mold

- **No spores or other fungal elements on tape samples**

- **Mold**
  - **Spores and other fungal elements on tspe sample**
  - **Remember" a single tape sample only tells you whether or not there was mold at that specific site.**
  - **It is NOT QUANTITATIVE**

Hypothesis #4 – No Exposure

Negative case

Extensive indoor/outdoor air sampling to document similar populations

- **Multiple matched samples indoors and out, preferably with windows and doors closed**
- **Spore samples (75 liters) microscopy**

Hypothesis #4 - Interpretation

No exposure

- Indoor and outdoor ppulations are similar in quality
- Indoor concentrations are lower than those outdoors (by taxon)

- Possible exposure
  - Fungal populations are different indoors and out
  - Pen/Asp is dominant indoors, absent or very low outdoors
  - Indoor concentrations for some spore types are higher by at least 10 fold than outdoors.

Hypothesis #5 – Hidden mold

Design a good air sampling strategy to document whether or not spore exposure is actually occurring

- Spore trap air samples near suspected hidden sources (microscopy) 75 liters
- Collect lots of samples
- Consider doing non-aggressive/aggressive pairs

Sample for fungal VOCs?

Hypothesis #5- Interpretation

No hidden mold:

- **No unusual types and/or concentrations of specific types on any air samples**

- **Hidden mold:**
  - **Unusual spore types and/or high conentrations of one or two spore types on at least one air sample.**

Hypothesis #6 – Visible Mold releasing spores/VOCS

Exposure can be assumed (no sampling)

Design a sampling strategy to document spore release from specific sources

- Surface (tape samples of visible mold (microcopy)
- Spore air samples near and far from visible growth and compare spore types

Hypothesis #6 - Interpretation

No spore release from visible growth

- Spore types on surface not present in air samples

- Minimal spore release from visible growth
  - Some surface spore types present in air close to growth but not further away (e.g., in the middle of the room, in another room)

Spore release and probable exposure

- Surface spore types abundant in air close to growth and also present distant from growth

Remember!!!

Exposure does not equal dose

Exposure is not sufficient evidence for cause/effect

The Data

- Ask for data In Excel format
- Print out the spreadsheets and include them in your notebook, or
- Store them in a separate data book or file and enter specific designations for each data set into the main notebook

Overall data interpretation

There are visible conditions that have or may lead to mold growth

There are odors likely associated with mold growth

There is visible mold in the occupied space

These are YES/NO interpretations based on visual inspection

Interpreting sampling data

Bulk and surface samples

- There is (or is not) mold growth on surfaces
- The types of mold growth present are:
- These data are not quantitative unless you
  - Collect samples from a measured area
  - Collect enough samples to document variability
  - Relate the sample data to the entire relevant space

Interpreting Air Sampling Data

Non activity/activity sampling

- Activity concentrations > an order of magnitude higher than non activity sample
- Spore types in activity samples similar to those found on bulk/surface samples
- Activity spore populations qualitatively similar to indoor rather than outdoor populations

Fungal Air Sampling Interpretation

Indoor/outdoor comparisons

- Accurate indoor/outdoor comparisons require the species name of the particles of concern
- This data is rarely (if ever) available
- Therefore, indoor/outdoor comparisons must be interpreted carefully, and should NEVER b the principal data documenting fungal growth or its absence.

Comparing indoor and outdoor air samples

MoldScore

- This statistic can be used to obtain a rough estimate of the likelihood of indoor growth given paired indoor/outdoor air samples.
- If your indoor sampling occurs over hours, new outdoor samples should be obtained

MOLD Range

Mold Range

This statistic gives you an idea about the distribution of outdoor mold concentrations and provides an estimate of whether or not your outdoor sample is unusual.

Thank you for your attention

If time permits I will be glad to answer questions now

Otherwise send questions to Dgallup@emlabpk.com

He will forward them to me, and I will answer within a week

Again, Thanks for attending