

# SAMPLING AND ANALYSIS

## **Environmental Sampling and Remediation**

# EVALUATING THE PROBLEM - ENVIRONMENTAL SAMPLING

- ◉ Characterize source
- ◉ Environmental variability
- ◉ Sampling
  - Bulk
  - Air
  - Tape
- ◉ There are many limitations to sampling

# INITIAL INVESTIGATION

- ◉ Site history and background
- ◉ Visual observation
  - Indoor and outdoor
  - sites of water and moisture
  - observed mold growth
  - “moldy odors”

# STRATEGY

- ◉ Visual inspection - good tool for assessing indoor moisture and fungal growth
- ◉ Sampling may be indicated when results of visual inspection are ambiguous or detailed information is necessary

# HUMAN HEALTH EFFECTS AND SAMPLING

- ◉ Dose response not well defined - concentrations in environment, species and human health responses all vary
- ◉ No universal agreement or recommended limits for fungi in indoor air

# MOISTURE TESTING

- ◉ Use of moisture detectors
  - probes
  - penetrating sensors
- ◉ Pro's and Con's
  - Relative measurement of moisture content  
moisture activity - no indication of mold growth  
only that moisture is present
  - Potential inaccuracies/false readings

# THERMAL IMAGING

- ◉ An useful tool to detect anomalies in building components. Water holds heat longer and results in water-damaged building materials retaining heat longer than adjoining or similar dry substrate.
  - Time saving
  - Examination of difficult to access areas
  - Examination of large areas quickly

# THERMAL IMAGING

- ◉ Disadvantages

- False readings

- Cold and warm air can create false positives and false negatives
    - Warm may mask wet materials by warming surface
    - Cold air can cause a thermal pattern indicative of moisture

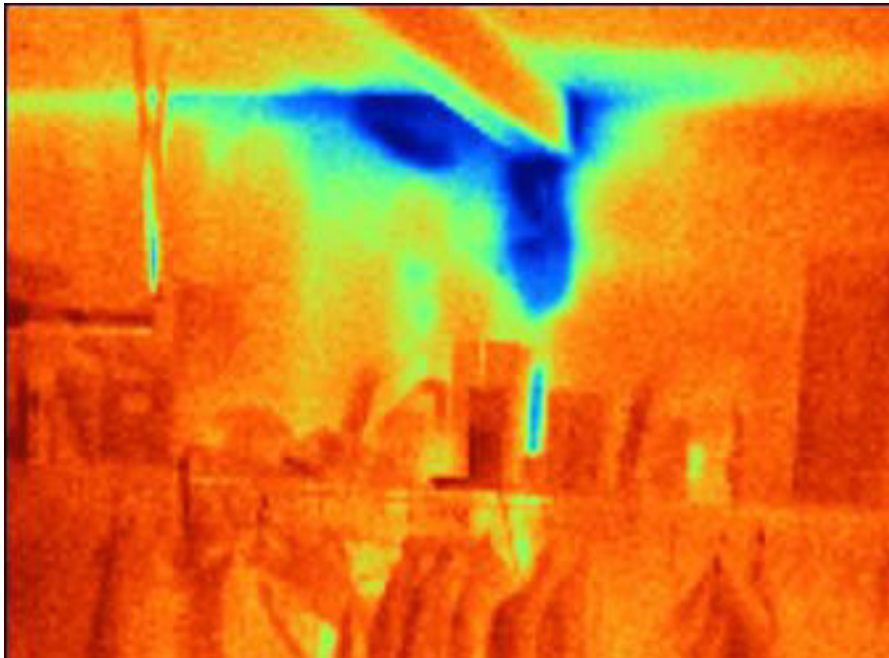
- Reflections can distort the thermal pattern

- Locating moisture behind ceramic tiles and some floor and wall covering can be difficult

- ◉ Use in conjunction with a moisture meter



# EXAMPLE OF AN IR IMAGE



# SAMPLING PROTOCOLS

- ◉ Sampling locations
  - Problem and non problem areas
  - Outdoor samples
- ◉ Data represents a moment in time
- ◉ Observe Site Conditions
  - Note weather, activity levels, furnishings, plants
  - HVAC operation
  - Accessibility to outside air - doors, windows

# DESTRUCTIVE TESTING IN WALL CAVITY

- ◉ Wall cavity is a potential reservoir for mold and moisture
- ◉ The presence of mold alone does not necessarily mean the occupied space is contaminated
- ◉ Opening wall cavities may create indoor contamination
- ◉ Opening wall cavity from the exterior

# WALL CAVITIES

## ◉ Inspections

- Snap shot of inside of wall only
- Molds on lumber surfaces is typical
- Spores from dusts, etc.

## ◉ Collecting air samples from inside

- Often done, typically not useful
- No comparison criteria (normal vs. abnormal)
- May lead to false indication of water intrusion/fungal growth



# SURFACE SAMPLING

- ◉ Tape, Swab or Bulk
- ◉ Direct microscopic exam
  - To determine and identify fungi on surface
    - Presence of mold spores
    - Mold growing or may have been growing
  - Indicator of a fungal reservoir
    - Marker genera - indicative of indoor mold growth

# SURFACE SAMPLING

## ◉ Disadvantages

- Not a direct indication of what might be airborne
- Sometimes results are interpreted wrong or poorly - may vary depending where sample is taken (i.e., dusty area)
- Not all the spores seen under the microscope may be viable (alive).
- It may be advisable to combine direct exam samples with culture methods to get a better picture of what live molds are present.

# SPORE TRAP MONITORING

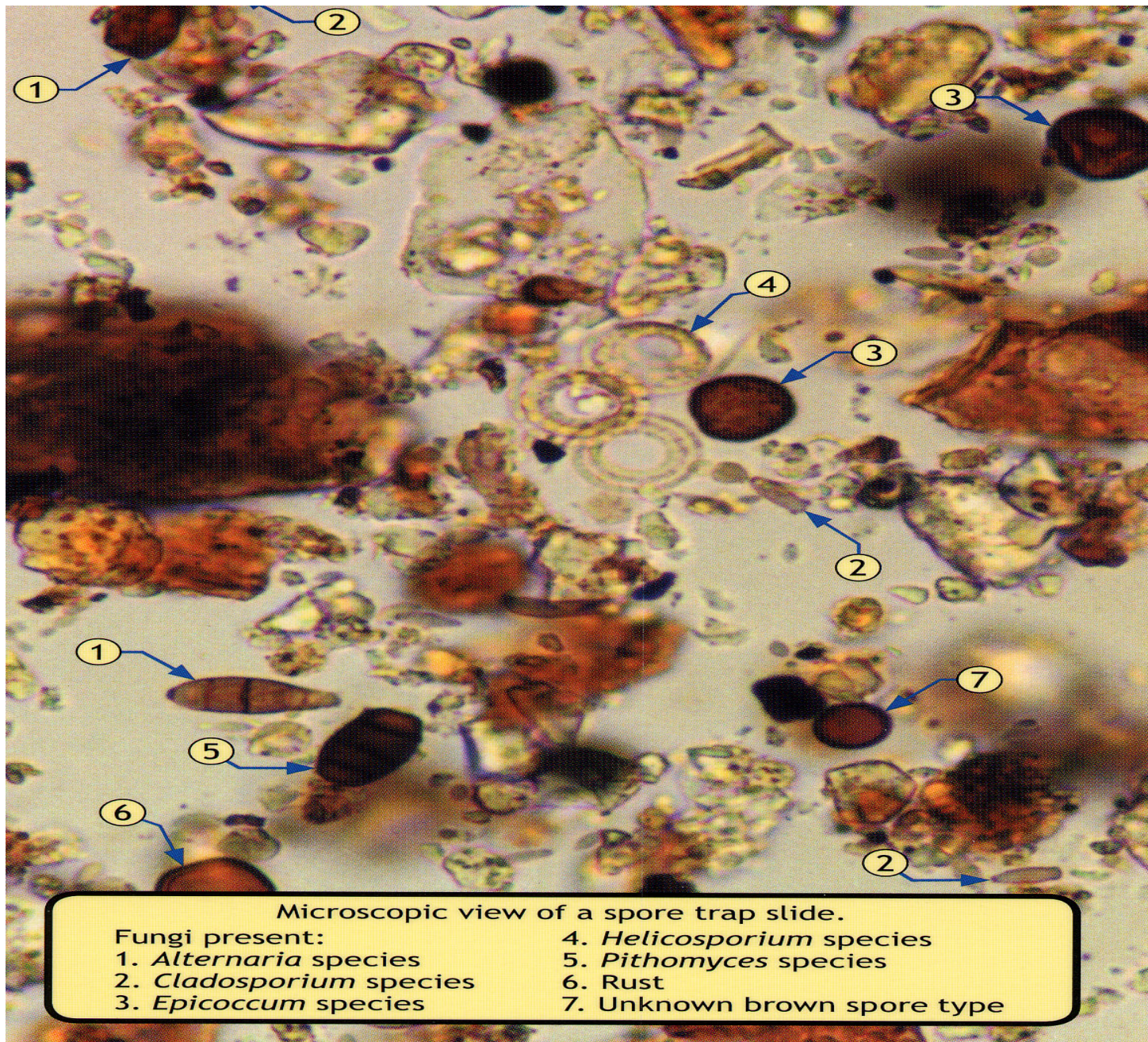
## ◉ Advantages

- Capture majority of spores/particulate matter in air
- Accurately characterize a problem
- Quantify pollen, skin cells, hyphal fragments, other particles

## ◉ Disadvantages

- Some spores difficult to identify - Pen/Asp
- Viability not assessed - not typically critical
- High variability
- Confounding issues - high concentrations not necessarily indicative of a problem
- Differences in interpretation







**SPORE TRAP REPORT: NON-VIABLE METHODOLOGY**

Instrument Used: Zefon Air-O-Cell volumetric air sampler

| Location: MB061799 –            | 4: Portable Bldg. #28,<br>afternoon, inside |               | 5: Portable Bldg. #28,<br>afternoon, inside |                | 6: Portable Bldg. #28,<br>afternoon, outside |                |
|---------------------------------|---------------------------------------------|---------------|---------------------------------------------|----------------|----------------------------------------------|----------------|
|                                 | raw ct.                                     | spores/m3     | raw ct.                                     | spores/m3      | raw ct.                                      | spores/m3      |
| Alternaria                      | 60                                          | 1,325         | 112                                         | 2,472          | 84                                           | 1,854          |
| Arthrinium                      |                                             |               |                                             |                |                                              |                |
| Ascospores*                     |                                             |               |                                             |                |                                              |                |
| Aureobasidium pullulans         |                                             |               |                                             |                |                                              |                |
| Basidiospores*                  |                                             |               | 4                                           | 88             | 8                                            | 177            |
| Botrytis                        |                                             |               |                                             |                |                                              |                |
| Chaetomium                      |                                             |               |                                             |                |                                              |                |
| Cladosporium                    | 3,115                                       | 68,764        | 6,440                                       | 142,163        | 5,740                                        | 126,711        |
| Curvularia                      |                                             |               |                                             |                |                                              |                |
| Drechslera/Bipolaris group      |                                             |               |                                             |                | 1                                            | 22             |
| Epicoccum                       |                                             |               | 1                                           | 22             |                                              |                |
| Fusarium                        |                                             |               |                                             |                |                                              |                |
| Myrothecium                     |                                             |               |                                             |                |                                              |                |
| Nigrospora                      |                                             |               |                                             |                |                                              |                |
| Other colorless                 |                                             |               |                                             |                |                                              |                |
| Penicillium/Aspergillus types†  | 4                                           | 88            |                                             |                | 40                                           | 883            |
| Pithomyces                      |                                             |               |                                             |                |                                              |                |
| Rusts*                          |                                             |               | 1                                           | 22             | 2                                            | 44             |
| Smuts*, Periconia, Myxomycetes* | 6                                           | 132           | 20                                          | 442            | 18                                           | 397            |
| Stachybotrys                    |                                             |               |                                             |                |                                              |                |
| Stemphylium                     | 1                                           | 22            | 2                                           | 44             | 2                                            | 44             |
| Torula herbarum                 |                                             |               | 1                                           | 22             |                                              |                |
| Ulocladium                      |                                             |               |                                             |                |                                              |                |
| Unknown                         |                                             |               |                                             |                |                                              |                |
| Zygomycetes (possible)          |                                             |               |                                             |                |                                              |                |
|                                 |                                             |               |                                             |                |                                              |                |
|                                 |                                             |               |                                             |                |                                              |                |
| Background debris (1-4+)††      | 3+                                          |               | 4                                           |                | 3                                            |                |
| Sample volume (liters)          | 45.3                                        |               | 45.3                                        |                | 45.3                                         |                |
| <b>TOTAL SPORES/M3</b>          |                                             | <b>70,331</b> |                                             | <b>145,275</b> |                                              | <b>130,132</b> |

**Comments:**

# CULTURABLE AIR SAMPLING

- ◉ Air drawn through an impactor plate with a petri dish below
- ◉ Spores incubate and grow on agar
- ◉ Viable fungi
- ◉ Indoor vs. outdoor air comparison
- ◉ Will differentiate species - *Pen/Asp*
- ◉ May be used for bacterial count

# CULTURABLE AIR SAMPLING

- ◉ Does not indicate non-viable spores/fragments
- ◉ 5-7 days for incubation
- ◉ Short sampling period (minutes)
- ◉ Fungi and bacteria present in the air may not be as well represented in culture - competition
- ◉ Some microbes do not grow well or at all on the culture media.

# CULTURABLE MOLD SPORE REPORT

Instrument Used: Andersen volumetric air sampler

| Location: MB061799 –    | 5: Portable Bldg. #28,<br>afternoon, inside |        | 6: Portable Bldg. #28,<br>afternoon, outside |          | BL:<br>Blank |        |
|-------------------------|---------------------------------------------|--------|----------------------------------------------|----------|--------------|--------|
|                         | raw ct.                                     | cfu/m3 | raw ct.                                      | cfu/m3   | raw ct.      | cfu/m3 |
| Acremonium              |                                             |        |                                              |          |              |        |
| Alternaria              | 2                                           | 35     | 5                                            | 88       |              |        |
| Aspergillus, other      |                                             |        |                                              |          |              |        |
| Aspergillus ochraceous  |                                             |        |                                              |          |              |        |
| Aspergillus flavus      |                                             |        |                                              |          |              |        |
| Aspergillus fumigatus   |                                             |        |                                              |          |              |        |
| Aspergillus nidulans    |                                             |        |                                              |          |              |        |
| Aspergillus niger       |                                             |        |                                              |          |              |        |
| Aspergillus versicolor  |                                             |        |                                              |          |              |        |
| Aureobasidium           |                                             |        |                                              |          |              |        |
| Beauveria               |                                             |        |                                              |          |              |        |
| Botrytis                |                                             |        |                                              |          |              |        |
| Chaetomium              |                                             |        |                                              |          |              |        |
| Cladosporium            | 195                                         | 13,039 | 200                                          | > 20,777 |              |        |
| Curvularia              |                                             |        |                                              |          |              |        |
| Drechslera              |                                             |        |                                              |          |              |        |
| Epicoccum               | 1                                           | 18     | 1                                            | 18       |              |        |
| Fusarium                |                                             |        |                                              |          |              |        |
| Mucor / Rhizopus        |                                             |        | 1                                            | 18       |              |        |
| Paecilomyces            |                                             |        |                                              |          |              |        |
| Penicillium             | 1                                           | 18     | 16                                           | 300      |              |        |
| Phoma                   |                                             |        |                                              |          |              |        |
| Sporobolomyces          |                                             |        |                                              |          |              |        |
| Stachybotrys            |                                             |        |                                              |          |              |        |
| Trichoderma             |                                             |        |                                              |          |              |        |
| Ulocladium              |                                             |        |                                              |          |              |        |
| Nonsporulating colonies | 8                                           | 141    | 15                                           | 283      |              |        |
| Sample volume (liters)  | 56.6                                        |        | 56.6                                         |          |              | No     |
| <b>TOTAL CFU*/M3</b>    |                                             | 13,251 |                                              | > 21,484 |              | growth |

\* cfu = colony forming units.

Comments:

Particle hole correction chart used for all calculations.

AIHA EMPAT NO.: 25518

# DATA INTERPRETATION

- ◉ Compare complaint and control samples
- ◉ Relative quantities
- ◉ Rank order of prevalence
- ◉ Unusual, allergenic types

# POLYMERASE CHAIN REACTION SEQUENCE DETECTION (PCR)

## ◉ Advantages

- Developed by EPA - uses fungal DNA
- Quick turnaround - 1 to 2 days
- Accurate fungal detection (whether viable or not)
- Will allow fungal differentiation where spore trap may not
- Good detection sensitivity
- Longer sampling period



# PCR

## ◉ Disadvantages

- Expensive compared to spore trap
- Not best for screening purposes as is spore trap or culturable (only detects fungi requested)
- Cannot distinguish viable from non-viable
- Currently not a large data base of indoor fungi
- No standards or guidelines - still must rely on indoor vs. outdoor results



# SAMPLING

- Often done by “mold inspectors”
  - Little basis in scientific validity
  - Data cannot be interpreted
  - Provides little information regarding conditions or fungal reservoirs
  - Sampling is conducted with no or incomplete objectives regarding what to do with the data

# WHEN AND WHY TO SAMPLE

- ◉ Only if it's done for a good reason
  - Demonstrate exposure when a health effect has already been established
  - Link to unknown reservoir
  - Post-remediation clearance sampling
- ◉ Know what you want from the data (hypothesis) before you design your sampling strategy
- ◉ Respect the limitations of the data
- ◉ Sampling is at times overdone with little forethought paid to interpreting the data