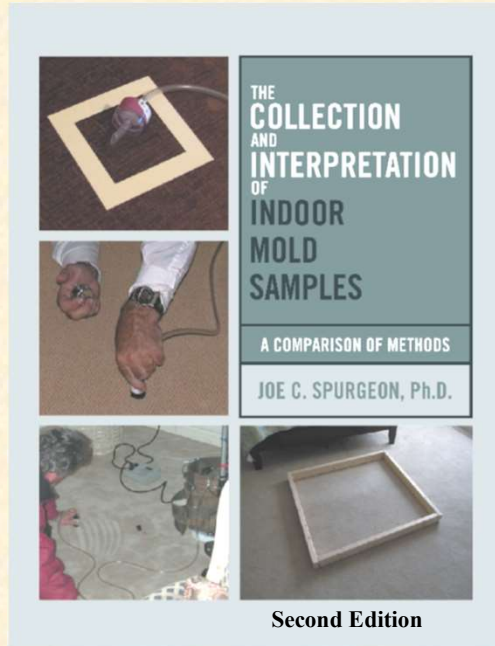


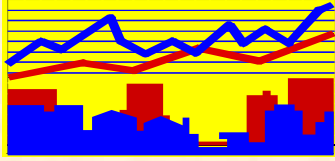
Soft Surface Samples

Part 1 Reporting Surface Dust Sample Results

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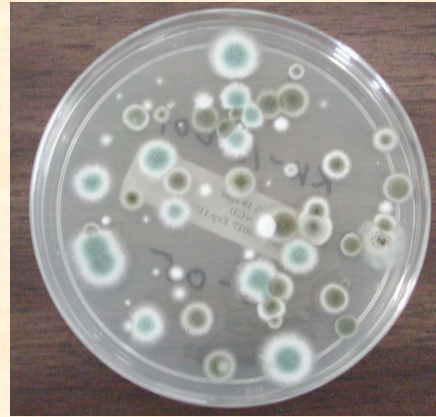


Introduction

Analyzing Surface Dust by Culturing

- Collected with swabs, filters, or Rodac plates
- Identified to species
 - Except *Penicillium* spp.
- Only detects culturable fungi
 - Viable (Living)
 - Grow on that media
- Incubation 7-10 days
 - some *Aspergillus* 14 days
- Semi-quantitative method
 - Colony Forming Unit (cfu)
 - Number of spores forming a colony is unknown
 - Heavy growth may result in low counts

Infrequently used today



cfu/mg or cfu/cm²

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Many of the samples discussed in this presentation were collected prior to the development of qPCR methods and were analyzed by culturing on MEA and/or DG-18 media. However, the concepts and logic illustrated in the discussions would apply to any method of analysis. Therefore, the reader should focus on those elements rather than the method of analysis unless that is the specific topic of discussion.

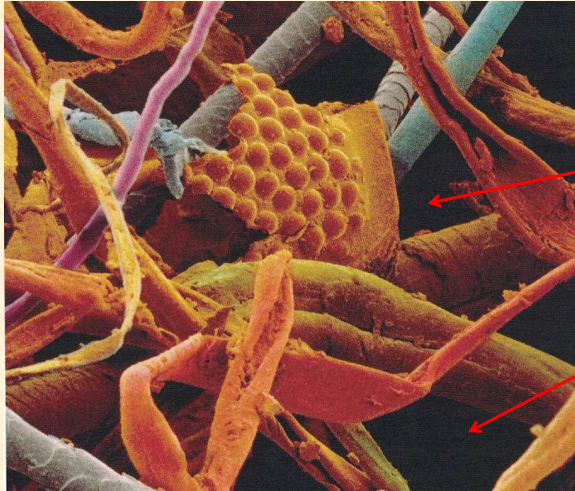
Analyzing Surface Dust by Microscopy

- Collected with tape lifts, swabs, or filters
- Identifies mold to genus or spore type
- Detects spores, hyphae, & phialides
- Lab reports “abundance”
 - Numerical scale
 - Descriptive scale



Tape Lifts on Soft Surfaces

IICRC Journal (1):4 (August, 2014)



Are there spores
below the fibers
that are never
sampled?

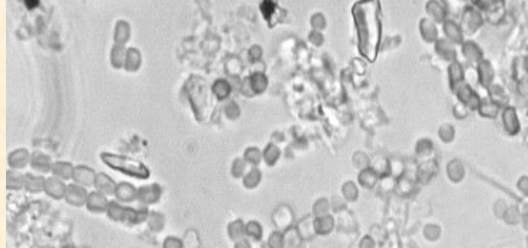
Swabs can at
least poke thru
the fibers

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A tape lift produces the best quality sample when used on a relatively clean, hard surface or to sample visible mold.

What the Analyst Would Like to See

Swab Sample or Clean Tape Lift



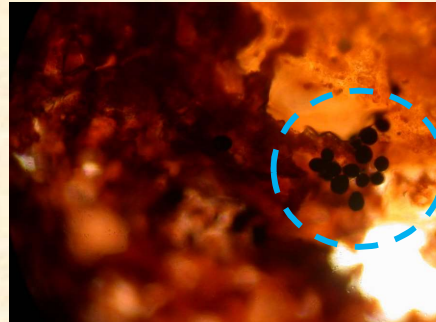
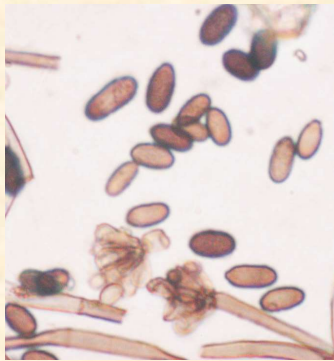
**Dry swab samples can be kept or archived
Wet swabs must be shipped immediately
(Don't collect on Fridays)**

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If you can see through the tape lift with ease, the analyst will probably be able to see any mold spores that were collected.

What the Analyst Often Sees on A Tape

**When are tape lift
samples most useful?
Clean, hard surfaces
Visible Mold**



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If you cannot see through the tape lift, neither can the analyst. Some spores can be seen, but probably not identified, nor counted in other areas of the tape lift.

qPCR v ERMI

- **Quantitative Polymerase Chain Reaction**
- **qPCR is a laboratory method for analyzing samples**

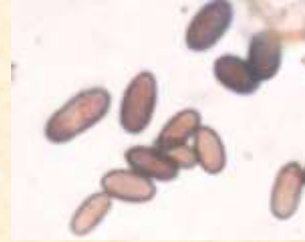
- **Environmental Relative Moldiness Index**
- **ERMI is a data-interpretation method for assessing the concentrations of 36 fungi in carpet dust samples**
- **Samples are analyzed by MSQPCR analysis**

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MSQPCR – Mold Specific Quantitative Polymerase Chain Reaction.

Spore-Equivalents v Spores

- Quantitative Polymerase Chain Reaction
- Results reported as spore-equivalents or cell-equivalents
- One cell = one spore-equivalent
 - 2 Stachy spores = 2 Sp-Eq
- Many cells = many sp-eq's
 - 2 Alternaria spores = 14 Sp-Eq?



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A spore-equivalent is not always the same as a spore. Even within the same qPCR lab report, the reported spore-equivalents for two species can indicate different spore concentrations. But this is just “technical”, qPCR is a very good lab method and a very useful inspector’s tool.

Analyzing Surface Dust by QPCR

- **MSQPCR (Mold Specific Quantitative Polymerase Chain Reaction)**
 - DNA analysis
- **Only detects fungal species when primers of species included in the analysis (pre-selected scans)**
- **Detects total (viable + nonviable) fungi**
 - Some spore and mycelia fragments also detected
- **Identifies to species (including *Penicillium* spp.)**
- **No overloading of sample, but some interferences**
- **Immediate analysis of sample, no incubation**
- **Quality of sample results can be lab-specific**

Sensitivity of qPCR v Culturing

Damp Crawl Space, Leaking Ducts

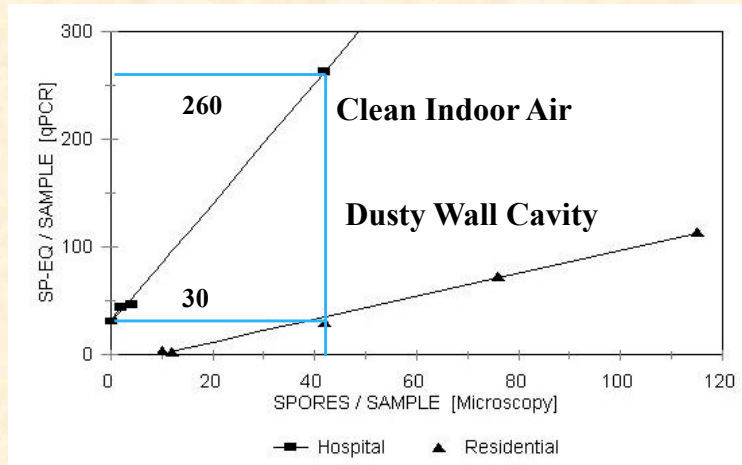
Total Fungi	SP-EQ / Sample	CFU/ Sample
SURFACE	qPCR	Culture
Air Return	2,066,000	3,400
Air Return	4,103,000	3,900
Air Return	4,015,000	19,200
Air Supply	9,430,000	14,300
Air Supply	46,200	16,000
Air Supply	9,601,000	26,200

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QPCR lab reports can contain some very large numbers compared to what an inspector is used to seeing. It's a matter of adjusting to them and not alarming the client when there is no need to do so.

qPCR *Asp/Pen* Spores: Different Environments

Dust can suppress qPCR results



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QPCR can have issues. The response can be suppressed by contaminants in the environment, like dust or Redwood. The point is that the two sets of data in the above graph are both useful (both nice straight lines), but samples collected in a clean environment cannot be directly compared to those collected in a dusty environment, for example.

Surface Mold v Airborne Mold

Tape Lift Samples from Hard Surfaces

Is Surface Mold Associated with Airborne Mold Spores?

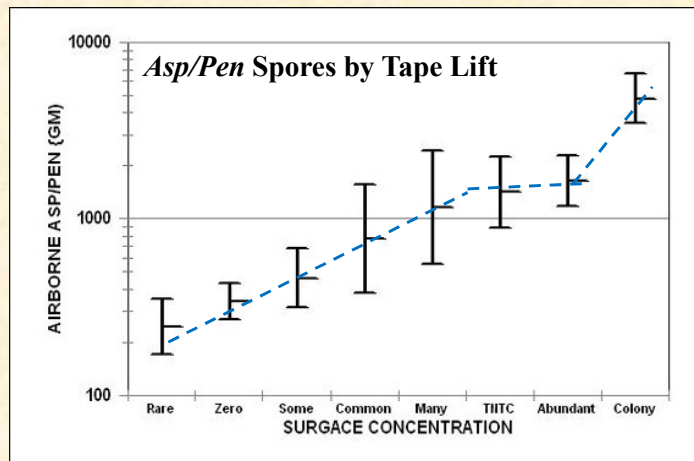
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Does surface mold contribute to occupant exposure? Is it a measure of Occupant Exposure Potential, or is it simply an indicator of Building-Related Contamination?

Airborne v Surface *Asp/Pen*

1,100 Tape Lifts and 240 Air-O-Cell Samples
Median Concentrations in the Same Room

Dr. Florence Wu; Aemtek, Inc.; Fremont, CA

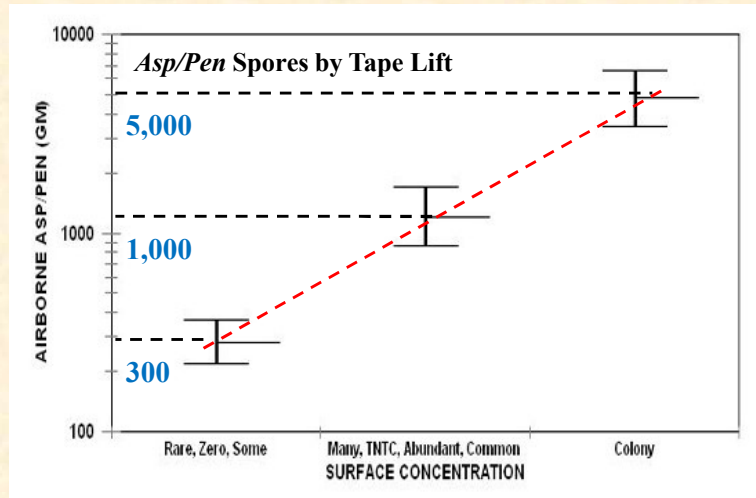


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These are the results for surface tape lift samples and Air-O-Cell samples collected in the same room. The median *Asp/Pen* concentration was calculated for each surface category.

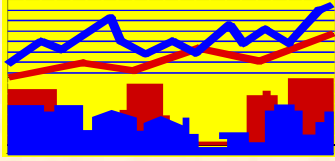
Airborne v Surface *Asp/Pen*

Dr. Florence Wu; Aemtek, Inc.; Fremont, CA



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There was a good association between the amount of surface mold in a room and the logarithm of the median concentration of *Asp/Pen* spores. Conclusion: Surface mold was contributing to airborne concentrations of *Asp/Pen* spores

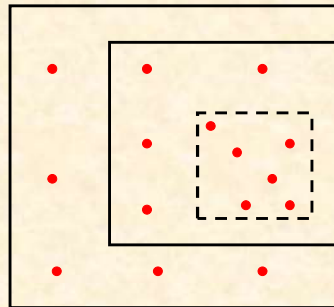


Reporting Laboratory Results

Problem with Sampling Surface Dust

Real-world Example: Failed Carpet Clearance

[Fungi are in the dust, not in synthetic carpet fibers]



- Clearance Criteria established
- Initial sample collected
- Carpet cleaned
- Clearance Failed
- Carpet cleaned again
- Clearance Failed
- Mold per unit weight of dust
 - Larger area of carpet
 - But the same mold/mg

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It was impossible to “clear” the carpet in this large office complex because the clearance criteria was established on a weight basis (Sp-Eq/mg of dust). Each time the carpet was cleaned a larger area of carpet had to be sampled to collect the same weight of dust (it was getting cleaner, so fewer Sp-Eq and fewer mg), but the dust contained the same amount of mold on a weight basis (same Sp-Eq/mg).

Methods for Reporting Surface Dust Samples

- 1. Weight-Analyzed Basis**
- 2. Total-Weight Basis**
- 3. Area-Sampled Basis**

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The method used by the laboratory to report surface dust samples can substantially affect the utility of the results for the Inspector.

Lab Method for Analyzing Surface Dust

- Laboratory “Standard Operating Procedure”
 - THE “SOP” IS IMPORTANT TO UNDERSTAND
- Results are reported on a Weight-Analyzed Basis
 - Use a 300 um sieve to remove large debris
 - Then select a 5-milligram portion of the “fine” dust
 - Discard the remaining dust
 - Analyze that 5-mg portion of dust for mold content
 - Divide the result by 5 and report
 - “Spore-equivalents per mg” of dust analyzed
 - BUT the number of mg in the sample is not reported

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Every mold inspector should know and understand their laboratory’s dust analysis SOP
– it’s critical to assessing your sample results !!

Method 1. Weight-Analyzed Basis

Lab Report	Surface 1	Surface 2
Weight Collected	Not Measured	Not Measured
Weight Analyzed	5 milligrams	5 milligrams
Fungi Detected	1,000 sp-eq/5 mg	1,000 sp-eq/5 mg
REPORT	200 sp-eq/mg	200 sp-eq/mg

Lab report indicates the level of Building-Related Contamination and/or Occupant Exposure Potential is the same for both surfaces

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The reported result of 200 sp-eq/mg ONLY refers to the 5 mg portion of dust that was analyzed. It does not inform the inspector of how much total mold there was in either of the samples. Therefore, the inspector has no way of assessing the two surfaces using the reported results.

How the Lab Reports the Results

“Reporting basis” affects the Inspector’s ability to assess both Building-Related Contamination and Occupant Exposure Potential

If the ”total weight” of a sample is not reported by the lab - Then the condition of the surface cannot be assessed accurately

BUT - the total weight of the collected dust will not be reported unless you request it

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If you requested the total sample weight, then you could calculate the amount of mold in each sample and could then compare and assess the conditions of the two surfaces.

Method 2. Total-Weight Basis

1. Request the lab weigh the “fine” dust after it is sieved
2. Multiply sp-eq/mg by the total number of milligrams in the sample
3. Report the result on a total-weight basis as sp-eq/sample

Lab Report	Surface 1	Surface 2
Weight Collected	400 milligrams	1,200 milligrams
Weight Analyzed	5 mg	5 mg
Fungi Detected	1,000 sp-eq/5 mg	1,000 sp-eq/5 mg
Sp-eq/mg	200 sp-eq/mg	200 sp-eq/mg
REPORT	80,000 sp-eq/Sample	240,000 sp-eq/Sample

Lab report indicates the Contamination and/or Occupant Exposure Potential is different for the two surfaces

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By requesting the lab weigh the dust sample, the total amount of mold in the sample can be calculated. This allows a better, more realistic comparison of conditions.

Total-Weight Basis

- **Still a potential problem with assessing Condition**
- **Were the 400 mg and 1,200 mg of dust collected from the same surface area?**
 - **If yes, then Condition of the surfaces may be compared**
 - **If not, then Condition cannot be compared**

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Although this is a better method, it's still not the best. It assumes the same area was sampled for both samples and this may not always be true.

Method 3. Area Basis

1. Inspector measures the area that was sampled
2. Requests lab report result on a Total-Weight Basis (sp-eq/sample)
3. Divide the reported result by the area sampled

Lab Report	Surface 1	Surface 2
Area Sampled	6" x 6"	12" x 12"
Weight Collected	400 milligrams	1,200 milligrams
Weight Analyzed	5 mg	5 mg
Fungi Detected	1,000 sp-eq/5 mg	1,000 sp-eq/5 mg
Sp-eq/mg	200 sp-eq/mg	200 sp-eq/mg
Sp-eq/Sample	80,000 sp-eq/Sample	240,000 sp-eq/Sample
REPORT	320,000 sp-eq/ft²	240,000 sp-eq/ft²

Condition of surfaces reversed in this example

Important: Now all samples collected may be compared

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Results reported on an Area Basis are standardized per unit area and can therefore be compared with other results. This is the only one of the three methods where that is true. Area Basis results are the only results that can be compared within projects, between projects, and between inspectors. This is critical to understand – stop and think about it !!

Area Basis

- **Health effects were better associated with the results when reported on an Area-Basis rather than a Weight Basis**
- **Ulrike Gehring, et al. $\beta(1\rightarrow3)$ -Glucan in House Dust of German Homes: Housing Characteristics, Occupant Behavior, and Relations with Endotoxins, Allergens, and Molds; Environmental Health Perspectives; 109: (2), February 2001**
- **Paul J. Liroy, et al. Dust: A Metric for Use in Residential and Building Exposure Assessment and Source Characterization; Environmental Health Perspectives; 110: (10), October 2002**

Conclusion: Results reported on an area basis are better for comparing Building-Related Contamination and/or Occupant Exposure Potential

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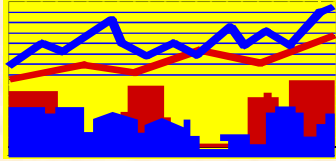
Reporting surface dust samples on an Area Basis provides data with the maximum utility for the mold inspector rather than the laboratory, and the optimal data for comparing and assessing condition.

Methods for Reporting Surface Dust

- **Weight-Analyzed Basis [Typical]**
 - Ignores the total weight of dust collected
 - Sample results are not standardized
 - Difficult to assess condition of the surface
- **Total-Weight Basis [Request]**
 - Based on the total weight of dust collected
 - But assumes the same sample area for all samples
- **Area Basis [Request; Preferred]**
 - Most unbiased assessment of Condition
 - Health effects were better associated with the results when reported on an Area-Basis compared to Weight Basis
 - Allows numerical assessment criteria to be established

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Take note of the last point. The Area Basis method allows numerical assessment criteria to be established. This topic as well as sampling methods will be discussed in Part 2.



Soft Surface Samples

Part 1

Reporting Surface Dust Sample Results

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