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The "CAP" Method for Assessing Surface Dust Samples for Mold

An Alternative to ERMI

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Presumption

- The assessment of Condition and/or Occupant Exposure Potential is influenced by the amount of fungi in the indoor environment
- Sensitized individuals?
  - Threshold concentration
  - "Trigger" a response

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The exposure potential typically increases for most occupants as the amount of contaminant mold in the indoor environment increases. However, sensitized or immune compromised individuals may exhibit a response when a threshold concentration is exceeded.

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Purpose

- A method for characterizing fungal concentrations in indoor dust samples using qPCR analysis
  - Based on fungal concentrations rather than a "score"
- A method that can be applied to all surface samples, not just carpets
- Method is cost-effective [2 – 4 – 8 - 14 fungi]
- Objective
  - Encourage broader use of qPCR
    - Lower-cost qPCR method applied to all surfaces
    - Assesses condition based on fungal concentrations

This presentation describes a qPCR-based method that is similar to ERMI, but the focus is on total fungal concentration rather than a calculated "score". In addition, this method can be applied to samples collected from any surface, not just carpets.

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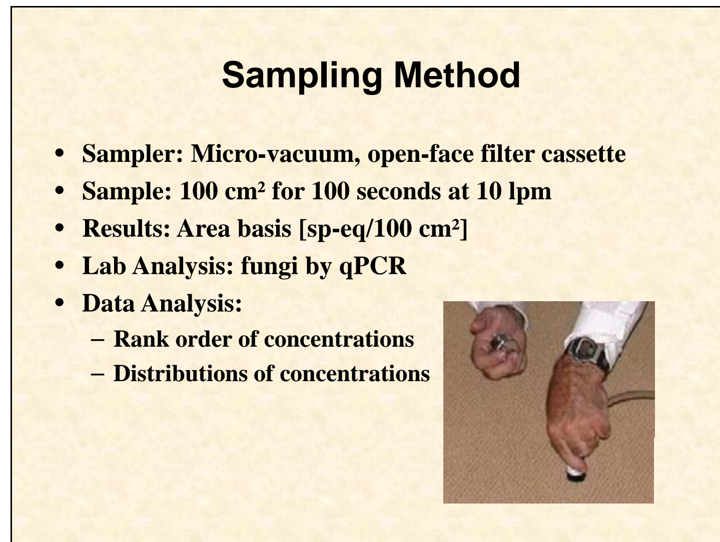

Previous studies have indicated that the open-face fixed area (OFFA) micro-vacuum method is an efficient [high sensitivity, low variability] sampling method for surface dust.

The area of the cassette is 4.9 cm^2, so sampling 20 spots represents an area of 98 cm^2 [essentially 100 cm^2]. A typical sample includes 20 spots that are sampled for 5-seconds each; and the results are reported as sp-eq/100 cm^2.

An airflow rate of 10 lpm for a 25 mm cassette is equivalent to 16 lpm for a 37 mm filter cassette [ASTM method for lead dust on floors].

Less than 5 mg of dust is typically collected in a sample. This allows the entire sample of dust to be analyzed by the laboratory; and the results are reported on a total-weight basis rather than a weight-analyzed basis.

The dust is analyzed using qPCR [DNA analysis], the same method used for ERMI and HERTSMI samples.

The sample results are interpreted using well-established, standard methods: rank order analysis or concentration distributions.

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90 Surface Dust Samples

Sample Matrix	Sample Size
Carpet Dust	39
Soft Surfaces	18
Air Supply Ducts	16
Air Returns	9
Hard Surfaces	8

Samples were analyzed for 36 ERMI fungi

The discussion was based on these 90 dust samples. The samples for each surface type were assessed independently.

Limitations of ERMI

Were ERMI scores associated with fungal concentrations?

No

The presentation titled “Limitations of the ERMI Method” [Part 1 of this presentation] demonstrated that ERMI scores did not increase as the fungal concentration increased. The same ERMI score was reported for samples in which fungal concentrations varied by a factor of 70 to 100. ERMI scores were not a good measure of “mold level”.

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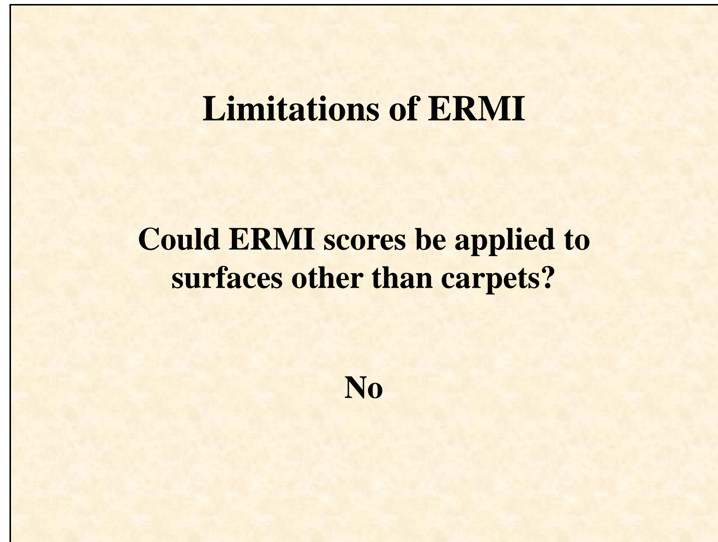

The presentation titled “Limitations of the ERMI Method” discussed why ERMI scores can only be applied to carpet dust samples; but not to couches, air ducts, door jambs, etc.

An Alternative Method To ERMI

How many fungal genera were sufficient to characterize the 90 surface dust samples?

The ERMI method requires 36 fungi to be analyzed, resulting in a substantial cost to the homeowner. If an equivalent method could be developed that required fewer fungi to be analyzed, then the same information could be obtained but at a lower cost to the homeowner. So how many fungi were required to provide the same information as provided by the 36 ERMI fungi?

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Prevalence of Dominant ERMI Fungi for 90 Samples

ERMI Fungi	Detection Rate
Clad type 1	100 %
Clad type 2	98 %
Clad herbarum	96 %
Aureobasidium	94 %
Eurotium	89 %
Alternaria	87 %
A. penicillioides	81 %
Epicoccum	80 %
A. niger	77 %
Mucor	73 %

These 10 fungi were detected most frequently in the 90 surface dust samples.
Example: *Eurotium* was detected in 89% of the samples, while *Mucor* was detected in 73% of the samples. The remaining 36 ERMI fungi not listed were detected at lower frequencies.
The point: An index based on fungi that are only detected in a small percentage of samples will not be very useful for assessing either condition or occupant exposure potential.

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Prevalence of Dominant ERMI Fungi for 90 Samples

ERMI Fungi	Total Count	% of Total
Aureobasidium	29,300,000	90.5 %
P. chrysogenum	674,000	2.08 %
Cladosporium type 1	643,000	1.99 %
P. brevicopmpactum	506,000	1.56 %
Clad sphaerospermum	275,000	0.85 %
Clad herbarum	228,000	0.70 %
Mucor	178,000	0.55 %
Alternaria	166,000	0.51 %
Cladosporium type 2	105,000	0.32 %
Epicoccum	63,000	0.19 %

SUM = 99.3%

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When the concentrations of *Aureobasidium* in each sample were added together, the total concentration in all 90 samples was 29,300,000 sp-eq. *Aureobasidium* accounted for 90.5% of the total concentration of fungi detected. In comparison, *Penicillium chrysogenum* [2nd most prevalent fungus] only accounted for 2.1% of the total fungal concentration detected in all 90 samples Together, these 10 fungi accounted for 99.3% of the total fungal concentration detected in the 90 samples. The remaining 26 ERMI fungi only accounted for 0.7% of the total fungal concentration.

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Percent of 36 ERMI Fungi

	Air Supply	Air Return	Carpet Dust	Soft Surface
Aureobasid	95%	87%	48%	64%
Clad type 1	1%	3%	41%	23%
SUBTOTAL	96%	90%	89%	87%
Pen (brev+chry)	0.2%	7%	4%	4%
TOTAL	96%	97%	93%	91%

Typically four fungi accounted for 91% to 97% of the 36 ERMI fungi

ERMI scores driven by *Aureobasidium* and *Cladosporium*

This table contains the percentages of the 36 ERMI fungi represented by the indicated fungal species.
Using carpet samples as an example. *Aureobasidium* accounted for 48% of the average total fungal concentration for the 39 carpet dust samples; and *Cladosporium* type 1 accounted for 41% of total fungi. Therefore, two fungi [*Aureobasidium* + *Cladosporium* type 1] accounted for 89% of the 36 ERMI fungi that were detected in the carpet samples.
When *Penicillium brevicompactum* and *Penicillium chrysogenum* concentrations were included, the four fungi accounted for 93% of the 36 ERMI fungi.

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CAP-4 Fungi in Rank Order

Fungi dominant in the 90 surface samples

Ranked by total concentration and frequency of detection

CAP-4 Fungi

- ***Aureobasidium pullulans***
- ***Cladosporium cladosporioides 1***
- ***Penicillium chrysogenum***
- ***Penicillium brevicompactum***

CAP: *Cladosporium – Aureobasidium – Penicillium*

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The 36 ERMI fungi were ranked by total concentration and frequency of detection for the 90 samples, identifying those fungi detected with the highest frequencies and at the highest concentrations – the dominant fungi.

For example, *Aureobasidium pullulans* ranked # 1 and *Penicillium brevicompactum* ranked # 4.

CAP is derived from the names of these dominant fungi.

Cladosporium – Aureobasidium - Penicillium

CAP-4 indicates that four fungi were included in the analysis; CAP-8 would indicate that eight fungi were included in the analysis, etc.

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CAP Fungi in Rank Order

CAP-4 Fungi [98%]

- *Aureobasidium pullulans*
- *Cladosporium cladosporioides 1*
- *Penicillium brevicompactum*
- *Penicillium chrysogenum*

CAP-8 Fungi [99.3%]

- *Aspergillus niger*
- *Eurotium amstelodami*
- *Aspergillus fumigatus*
- *Stachybotrys chartarum*

CAP-14 Fungi [99.9%]

- *Aspergillus flavus*
- *Aspergillus penicillioides*
- *Aspergillus versicolor*
- *Chaetomium globosum*
- *Trichoderma viride*
- *Wallemia sebi*

CAP-2 Fungi [96%]

- *Aureobasidium pullulans*
- *Cladosporium cladosporioides 1*

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Depending on the objective of the inspection, the mold inspector may suggest that 2, 4, 8, or all 14 fungi be included in the laboratory analysis; with progressively higher costs to the homeowner.
CAP-2 accounted for 96% of the total concentration of the 36 ERMI fungi, while CAP-14 accounted for 99.9% of the fungal concentration.
CAP-8 includes the CAP-4 fungi plus *A. niger, E. amstelodami, A. fumigatus,* and *S. chartarum*. CAP-14 includes the CAP-8 fungi plus the additional fungi listed. CAP-2 is only intended for an exploratory, low-cost assessment of an indoor environment.

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Assessing Indoor Environments

- **Exploratory Cost-constrained**
- **Assess condition of the indoor environment**
- **Assess condition plus potential for exposure**
- **Assess condition plus occupant exposure potential**

- **CAP-2**
- **CAP-4**
- **CAP-8**
- **CAP-14**

CAP-2 would be used for a low-cost, initial survey of a space; only used for a preliminary assessment of the condition of the surface sampled.
CAP-4 would be the basic, default method for assessing condition.
CAP-8 would be used for assessing condition plus a basic assessment of occupant exposure potential.
CAP-14 would be used for assessing both condition and occupant exposure potential.

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Were CAP fungi representative of ERMI fungi?

When the objective is assessing the condition of the surface sampled, can the CAP-8 method provide information that is equivalent to the ERMI-36 method?

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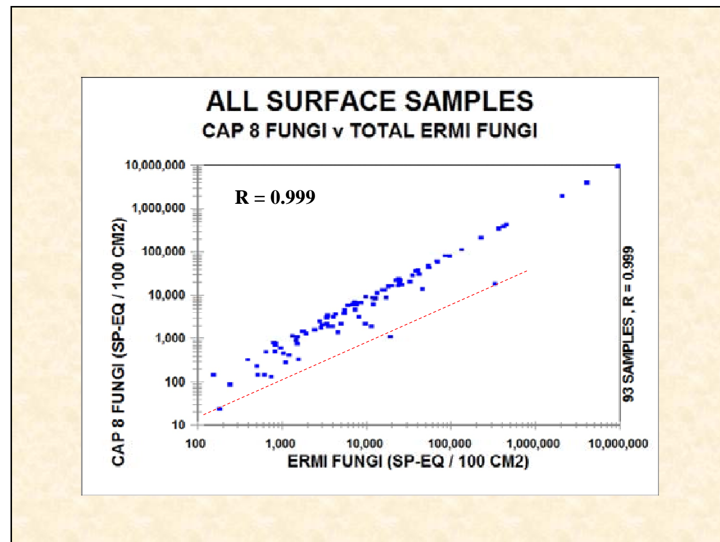

This graph did not differentiate between surface type; all 90 surface dust samples were included in the correlation.
The comparison was between CAP-8 fungi and the 36 ERMI fungi. The good correlation suggested that CAP-8 was an effective measure of ERMI-36; so the lower-cost CAP-8 method provided the same information as the more expensive ERMI method for assessing condition.
First, CAP-8 was a good measure of condition. The three samples that deviated from the main correlation (red dashed line) were due to *Aspergillus penicillioides* and *Aspergillus versicolor* (so these fungi should be included in an assessment).
Second, a comparison for CAP-14 (assessing condition plus occupant exposure) was an even better estimate for ERMI fungi.

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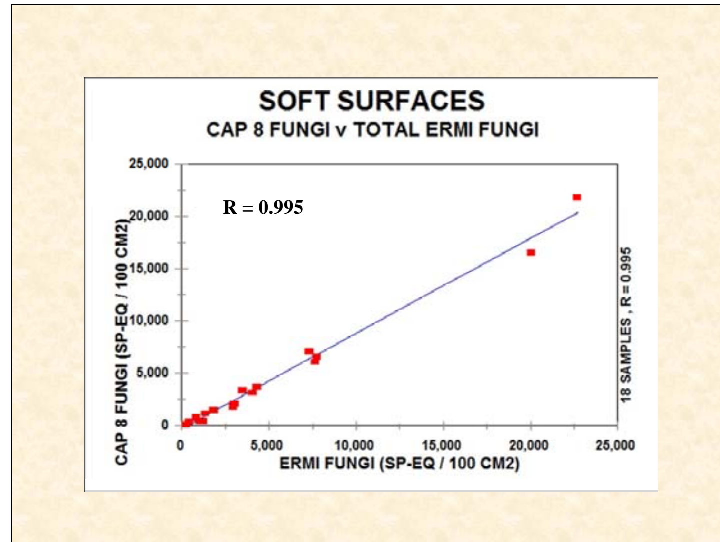

This graph showed the agreement between CAP-8 fungi and ERMI-36 fungi for a specific surface type (soft surfaces). Again, the lower-cost CAP-8 fungi were in good agreement with (were a good predictor of) ERMI-36 fungi.

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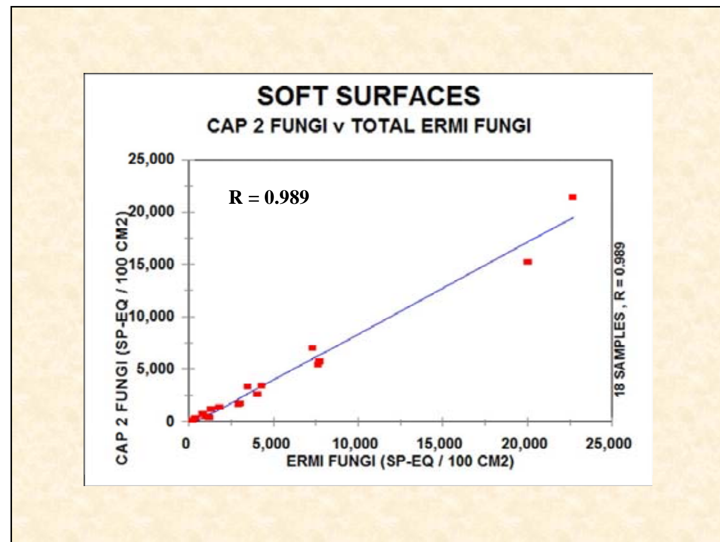

This graph showed the agreement between CAP-2 fungi and ERMI-36 fungi for soft surface samples. Even CAP-2 fungi were in good agreement with (were a good predictor of) ERMI-36 fungi. CAP-2 only includes *Aureobasidium pullulans* and *Cladosporium cladosporioides* type 1 fungi. These two fungi, by themselves, dominate the ERMI-36 fungi.

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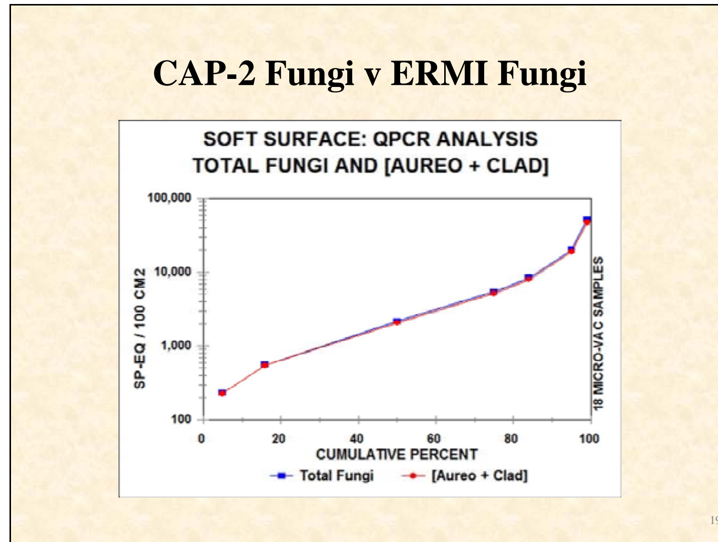

This graph is the “log plot” for the previous graph; concentration versus cumulative percentile. These are the distributions for CAP-2 fungi (red) and ERMI-36 fungi (blue). They are essentially the same distribution; which supports the previous statements that even CAP-2 fungi provided a good assessment of the condition of the surface sampled.

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CAP Fungi v 36 ERMI Fungi

Coefficient of Correlation

SURFACE TYPE	CAP-8	CAP-2
Carpets	0.988	0.983
Soft Surfaces	0.995	0.989
Hard Surfaces	0.999	0.999
Air Supply Ducts	0.999	0.999
All Surfaces	0.999	NA

CAP-2 fungi estimated total ERMI fungi as well as CAP-8 fungi

A good correlation was obtained for the five surface types listed in the table. Therefore, for all surface types, either CAP-2 or CAP-8 could be used to provide a good estimate of the condition of the surface that was sampled.

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Is there a rational method
for distinguishing between
"normal" and "amplified" conditions?

Percentile Concentrations

When assessing the condition of an indoor surface, how can we determine if the result reported for a surface dust sample is acceptable or unacceptable? The following discussion is based on the use of cumulative percentiles to interpret sample results. For example, what is the meaning of the "75th percentile concentration"?

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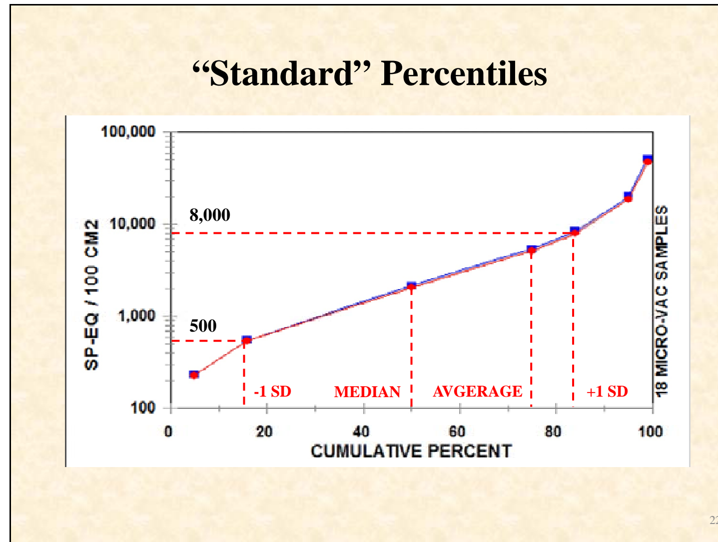

The median concentration is the 50th percentile and the average concentration is roughly the 75th percentile. The 16th and 84th percentiles are one standard deviation below and above the median concentration.

Cumulative percentiles can be easily determined from the above graph. For example, if the laboratory reported a result of 500 sp-eq, we can locate that concentration on the vertical axis, and the horizontal axis would indicate that concentration was about the 15th percentile concentration. We would only expect 15% of sample results to have a lower concentration (85% would have a higher concentration). So, this would be a low concentration compared to the 18 similar indoor environments represented by this graph (acceptable).

if the laboratory reported a result of 8,000 sp-eq, we can locate that concentration on the vertical axis, and the horizontal axis would indicate that concentration was about the 85th percentile concentration. We would only expect 15% of sample results to have a higher concentration (85% would have a lower concentration). So, this would be an elevated concentration compared to the 18 similar surfaces represented by this graph (unacceptable).

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Decision Logic: Percentile Concentrations

Examples of "rational" interpretations

Percentile Concentration	Decision Logic
80th %-tile and above	Assessment: Unacceptable
70th – 79th %-tile	Possibly Contaminated
69th %-tile and below	Assessment: Acceptable

This is just one example of a decision logic for interpreting sample results. It is no better than other decision logics; it is simply an example of a logic in which the decision criteria are associated with the "standard percentiles".

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Interpreting Sample Results

- 1st: CAP is based on total fungal concentrations rather than a differential "score"
- 2nd: CAP interprets sample results by referencing them to cumulative percentiles
  - The "distribution of concentrations"
- 3rd: CAP results are interpreted relative to a particular surface type
  - Carpet, soft surface, air return, etc.

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ERMI scores are calculated by subtracting one group of fungi from a second group as a measure of "mold level".
The CAP method adds both groups of fungi together to obtain a measure of "mold level".
In practice, ERMI does not differentiate between samples collected from various surfaces.
The CAP method can be applied to all surface dust samples.
CAP samples are interpreted based on cumulative percentiles rather than a "score".
For example, what is the significance of a reported concentration of 8,000 sp-eq/100 cm^2?

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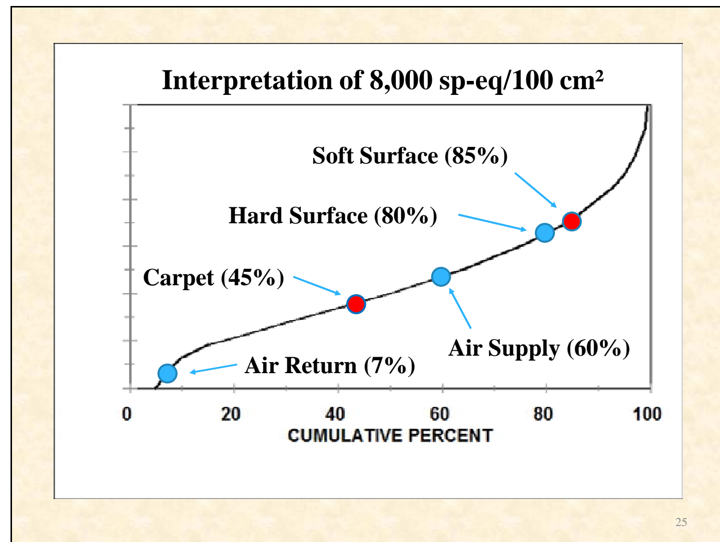

A reported result of 8,000 sp-eq cannot be interpreted without reference to the surface type that was sampled. For example, it is the 7th percentile (normal) if collected from an air return, but the 85th percentile concentration (amplified) if collected from a soft surface item. This limitation applies to any surface dust sample, even ERMI samples collected from surfaces other than carpets.

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Distributions: *Stachybotrys*

	SP-EQ / 100 CM²		
%-tile	Carpet	Air Supply	Air Return
95th %-tile	249	1,910	6,718
84th %-tile	93	495	1,863
75th %-tile	57	251	977
50th %-tile	20	61	255
16th %-tile	4	8	35
5th %-tile	2	1	10

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What about a sample result of 250 sp-eq/100 cm² of *Stachybotrys*? If the sample was collected from a carpet, it would be the 95th percentile, the 75th percentile if collected from an air supply, and the 50th percentile concentration if collected from an air return. The surface type matters.

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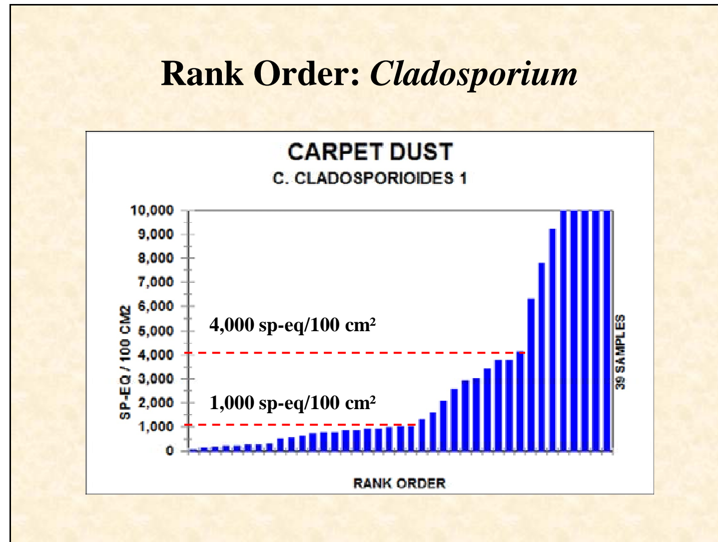
Is there a simple method for distinguishing between "normal" and "amplified" fungal concentrations?

Rank Order Analysis

Rank order analysis is very simple, but it can be an effective method for assessing the significance of sample results. A rank order analysis is performed by simply listing a series of sample results from lowest to highest concentration, then making a graph of the ordered concentrations.



For example, assume a number of sample results are available for *Cladosporium* concentrations in carpet dust, as in the above graph. The sample results were listed from lowest to highest concentration and then simply graphed. By just looking at the graph, there are some obvious breaks in the data at about 1,000 sp-eq and 4,000 sp-eq. One might then assume that concentrations less than 1,000 sp-eq were representative of "normal" carpets, while concentrations greater than 4,000 sp-eq were indicative of "amplified" conditions.

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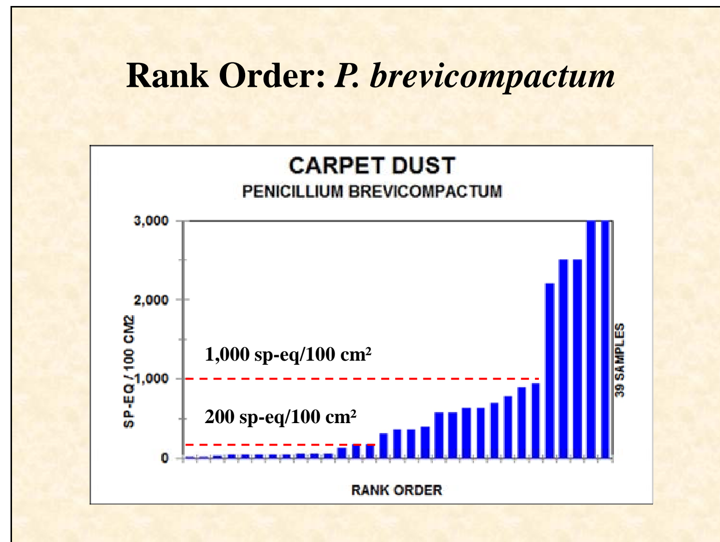

These data are for *Penicillium brevicompactum* concentrations in carpet dust samples. There are some obvious breaks in the data at about 200 sp-eq and 1,000 sp-eq. One might initially assume that concentrations less than 200 sp-eq were representative of “normal” carpets, while concentrations greater than 1,000 sp-eq were indicative of “amplified” conditions.

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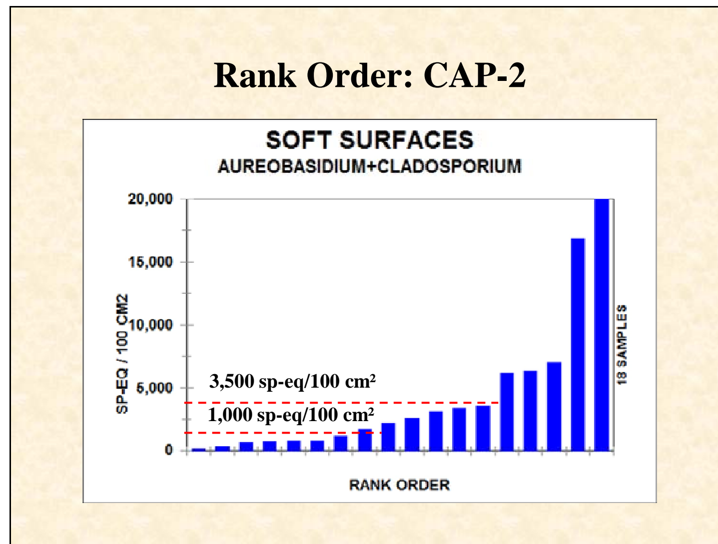

These are CAP-2 data for soft surfaces. There are breaks in the data at about 1,000 sp-eq, 3,500 sp-eq, and 7,500 sp-eq. Concentrations less than 1,000 sp-eq were probably representative of “normal” conditions, but the limit for “amplified” conditions is less clear. Is it 3,500 sp-eq or 7,500 sp-eq? Deciding to use either of these concentrations as being indicative of “amplified” conditions would be a “best guess”, but at least the range of transitional concentrations was identified.

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Distributions: CAP-2 & PEN-2

Soft Surfaces	SP-EQ / 100 CM²	
PERCENTILE CONCENTRATION	[Aureo + Clad]	[P. Brev + P. Chry]
95th %-tile	19,000	2,700
84th %-tile	8,000	425
75th %-tile	5,000	170
50th %-tile	2,000	25
16th %-tile	500	2
5th %-tile	200	NA

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These are the calculated cumulative percentiles for the same soft-surface samples discussed in the previous slide. The CAP-2 distribution indicated that the 75th percentile concentration was actually 5,000 sp-eq/100 cm². In comparison, the simple rank order analysis suggested the transition occurred between 3,500 and 7,500 sp-eq/100 cm² (a rather good agreement between the simple and more complex methods).

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Summary of CAP Method

- CAP is lower cost than ERMI
  - 2, 4, 8, or 14 fungi
- Sampling method is user-friendly
  - Micro-vac filter cassette
- Method may be applied to any surface dust sample
  - Not just carpets
- Sample results associated with fungal concentration
- Guidance based on standard methods
  - Rank order
  - Concentration distributions

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Final Thoughts

The certainty that numerical results for mold samples cannot be interpreted may be a false notion

The rank order method is very simple. Anyone could actually be using it in less than an hour.

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