Condition 2 Settled Spores: Case Studies Examine a Method to Improve Their Detection and Removal

by William M. Vaughan, PhD, QEP, CIEC

With the IICRC S520-2008 Standard and Reference Guide for Professional Mold Remediation (S520-2008) calling for cleanup of Condition 3 (actual mold growth) and Condition 2 (settled spores), the question is how to most effectively identify Condition 2 contamination and how to most effectively remove these settled spores. This paper presents an approach developed to characterize settled spores via “paired” air samples — quiet and fan disturbed — using a common 12-inch fan. The 12-inch fan stirs up the settled spores from a suspect area and mixes/integrates them so they can be sampled via spore trap. If comparison of the results from the paired samples shows a statistically significant difference between the quiet and the fan-disturbed, one can approach the remediation as a Condition 2 issue.

This is not a research project, or a controlled study, but a discussion of field data. It includes small sample sizes, an unavoidable limitation of the study. Five case studies are presented where this approach has been used. The case studies will demonstrate that settled mold spores are not removed by the methods many remediation contractors have used.

After HEPA vacuuming, an initial spore trap air sample can show low spore levels, yet the area may still be contaminated by settled spores which are not detected by the traditional (quiet) air sampling method: setting up a tripod with a spore trap and drawing air into/onto the collection medium. This approach detects spores that have not yet settled and the results may be misleading. In many situations where “visual inspection” and quiet sampling led the mitigator to conclude that conditions were acceptable and the initial remediation effort sufficient, paired sampling demonstrated it was not.

Visual inspection is not adequate for detecting settled spores, since they are below the resolution limit of the human eye, about 40μ (microns)¹, nor is quiet sampling. Settled spores have been a critical element in IICRC’s approach to mold remediation since the release of the initial S520 in 2003, spurred by knowledge of the potential health impact of molds and the fact that mold spores are biological organisms, capable of recontaminating a space. In contrast to an abatement project, where if you were to remove 99.9 percent of contaminants such as oil, PCB or lead, the remainder would forever be 0.1 percent of the original contamination, if you removed 99.9 percent of mold spores and a leak recurred, there is a good chance that the 0.1 percent of settled spores that remained would help facilitate
full recontamination, since mold spores amplify in response to moisture.

An alternative remediation method called “air polishing” is also presented. Following thorough HEPA vacuuming in a space, air polishing involves a combination of aggressive air disturbance with strong fans/leaf blowers and several oscillating fans in combination with air filtration units (AFUs), commonly called air scrubber(s), to suspend and remove most of the settled spores. A case study of air polishing is presented, including the post-mitigation paired-sampling results.

**Sampling Methods Compared**

Some Indoor Environmental Professionals (IEPs) recommend a tape lift or swab samples to detect the presence of settled spores, Condition 2 contamination, as part of an initial baseline assessment or post-remediation verification (PRV). But where are those samples to be taken? Floors? Walls? Easy-to-reach places? Every stud bay? Every fifth stud bay? There is no recognized standard.

Another consideration is how many surface samples you need from each location of concern. Dr. Harriet Burge (Indoor Environment Connections, October 2009) suggested that “to obtain an even marginally representative sample, at least 10 percent of the surface in question must be sampled.” This raises the very real question of whether the desire for scientific confidence in the results will be compromised by real-world budget constraints.

A different approach is to take “disturbed” air samples. My company has utilized this approach since 2004. As will be shown, it has been both technically effective and cost effective (when compared to surface sampling, the savings are in excess of 90 percent) in addressing Condition 2 mold contamination.

The advantage of disturbing the air is that settled spores from many surfaces in an area, including rafters in cathedral ceilings, nooks and crannies, and baseboard heat exchanger fins, can be impelled into the air and mixed so that they can be sampled via a spore trap. If comparison of the results from the paired samples shows a statistically significant difference between the quiet and the fan-disturbed, one can approach the remediation as a Condition 2 issue.

Five case studies are presented where this approach has been used. The case studies demonstrate that settled mold spores are not removed by the methods many remediation contractors have used.

After HEPA vacuuming, an initial spore trap air sample can show low spore levels, yet the area may still be contaminated by settled spores which are not detected by the traditional (quiet) air sampling method. This approach detects only those spores that have not yet settled and the results may be misleading. In many situations where “visual inspection” and quiet sampling led to the conclusion that conditions were acceptable and the initial remediation effort sufficient, paired sampling demonstrated it was not.

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The advantage of disturbing the air is that settled spores from many surfaces in an area, including rafters in cathedral ceilings, nooks and crannies, and baseboard heat exchanger fins, can be impelled into the air and mixed so that they can be sampled using spore traps. The disturbance integrates settled spores from many possible locations in an area. The disturbance simulates, to some extent, human activities that could remobilize the settled spores to travel to uncontaminated areas after containment barriers are removed.

An alternative remediation method called “air polishing” is also presented. Using the paired sampling approach as a model, an “air polishing” process can be utilized to improve on the overall remediation effort after normal HEPA-filter cleaning of surfaces.

The use of strong leaf blowers at the air polishing stage — not at the initial, preremediation, baseline investigation stage — aims at aggressively moving any settled spores into the air. Several oscillating fans minimize the quiet zones and keep the spores airborne longer for filtration by the air filtration units (AFUs). After a few rounds of relocating the exhaust from AFUs, oscillating fans and additional leaf blowing, far fewer settled spores will be left over, resulting in a high likelihood of a successful PRV evaluation and fewer return trips that extend the remediation. This will save money for the client and shorten the overall remediation while achieving documented success — a cleaner space.
that they can be sampled using spore traps. The disturbance integrates settled spores from many possible locations in an area. The disturbance simulates, to some extent, human activities that could remobilize the settled spores to travel to uncontaminated areas after containment barriers are removed. Those activities might include:

- fans set up to cool occupants or provide general ventilation.
- installation of carpet during which rolls of carpet and pad are dropped onto the subfloor and rolled out, creating sudden strong puffs of air.
- opening windows or operating attic fans that bring in outdoor drafts.
- remodeling activities that involve dropping supplies or material, such as drywall panels, onto the floor.
- vacuuming floors, carpets, furniture, woodwork, etc. in the normal course of home maintenance.

During these “normal” human activities, settled spores can be resuspended into the air by air currents, vibrations or occupant activities, then slowly settle onto the new/cleaned surfaces after refurbishing. The objective is to simulate occupant activities using a typical table fan to make sure the remediation activity to date has been reasonably effective, and if not, suggest further remediation efforts to reduce the settled spores to an acceptable level.

**Disturbed Air Sampling Methodology**

In early 2004, we adopted the practice of the asbestos abatement industry where leaf blowers are operated and moved about within containment areas for extended periods of time before and during air sampling. These aggressive “clearance” samples are called for under U.S. EPA regulations and must result in very low fiber counts on filter samples before an area is declared clean enough to be released for occupancy.

This approach for mold was criticized by clients and remediators, although it did point out areas where additional remediation was required. The criticism was that the extremely strong drafts created by leaf blowers were not typical for lived-in spaces and resulted in too stringent a remediation challenge in light of the absence of firm remediation guidance.

The protocol was modified to using 12-inch household fans, either table or stand styles. These are the type and size fans commonly used to provide ventilation and cooling in homes all over the country. The lighter level of disturbance was acceptable in a living space and was not extreme, as the leaf blowers were. The fan assembly is removed from its base and a technician disturbs the air by directing the fan around the space using a back and forth motion in a regular grid pattern across moldings and horizontal ledges, into baseboard fans, into corners, at ceiling rafters — anywhere where spores might have settled (Illustration A) — then the steps are repeated in a perpendicular pattern. Three to five minutes is usually sufficient to disturb the surfaces in a standard room-sized space.

Quiet air samples are collected in combination with disturbed air samples, and the difference in the concentration of total spores is used to assess Condition 2 contamination. In this way, two samples, usually spore trap samples, give an integrated picture of mold spore contamination in an area. By using total airborne fungal spores (as opposed to “culturable fungi”), one is best able to focus on the health impact for occupants, since both viable and nonviable spores can cause allergic reactions in sensitized individuals. (Note: It is prudent but not required that at least one outdoor air sample be taken during these efforts in order to compare the mix of outdoor spores with those indoors to demonstrate any water-induced mold amplification.)

**Sampling Case Studies**

Following are five representative case studies that describe the application of the paired sampling method, and the improved results obtained with this method.

**Example 1:** Failure of two baseboard heaters led to damage from direct water...
as well as condensation. Four separate areas in the house (A–D) were contained and remediated by HEPA vacuuming and wiping down. Post-remediation verification (PRV) sampling was done at a time when the outdoor total spore level was 2,650 S/m². The same work crew worked all areas.

Discussion: Chart 1 shows the initial post-remediation air sample pairs (quiet and disturbed) in each of the four areas.

- In areas A and C, disturbing the air with the 12-inch fan stirred up few additional spores.
- In area B, adjacent to one of the leaks, the total spores were measured at 1,222 S/m² using the quiet sampling method, a seemingly acceptable level compared to the 2,640 S/m² in the outdoor air. However, the total spores increased 18-fold when the disturbed air sampling method was used, and within those samples, the Aspergillus-Penicillium (Asp-Pen) spores increased 24-fold (i.e., well over an order of magnitude) indicating that substantial numbers of settled spores persisted after traditional remediation had been done.
- In area D, on the floor below the other leak, the difference in levels induced by the fans’ drafts is even more dramatic. The quiet sampling, at 944 S/m², again seems acceptable. However, the total spore levels increased 41-fold when measured using the disturbed air sampling method, with Aspergillus-Penicillium spores increasing some 120-fold!

Conclusion: Disturbed air sampling increased the concentration of Aspergillus-Penicillium spores 24-fold in area B and 120-fold in area D, but did not result in an increase in concentration in areas A or C. The additional information from a disturbed air sample can point to the need for initial or further remediation to reduce Condition 2 contamination.

Example 2: A rental apartment with a leak in the bathroom above the kitchen. A handyman had removed part of the kitchen ceiling. A plumber fixed the leak. No mold remediation professional was involved. The tenant wanted air sampling to confirm conditions were safe to reoccupy the apartment following those “nonprofessional” repairs.

Discussion: The kitchen looked clean. There was no visible mold growth (VMG) and no moldy odor. However, as illustrated in Chart 2, disturbed air sampling detected substantially higher concentrations of both indicator spores and total spores compared to quiet sampling. Under quiet sampling:
- Total spores were less than 400 S/m³, with only 267 S/m³ being the leak indicator Aspergillus-Penicillium (Asp-Pen) spores.
- No Stachybotrys (Stachy) spores were detected.

Under disturbed sampling:
- Substantially higher concentrations of both indicator spores and total spores were found compared to quiet sampling. Total spores increased to almost 30,000 S/m³ with Aspergillus-Penicillium spores at nearly 14,000 S/m³.
- Nearly 4,400 S/m³ Stachybotrys spores were detected (Chart 2).

The paired sampling approach detected the presence of settled contaminant spores that were not detected by quiet sampling alone. Also, the substantial increase in Stachybotrys spores suggests that the spores were “dry,” and probably residual contamination rather than current “wet” growth.

Conclusion: Disturbed sampling resulted in a 52-fold increase in Aspergillus-Penicillium spores compared to quiet sampling and the detection of a substantial concentration of Stachybotrys spores which were not detected by quiet sampling.

Example 3: An intermittent leak from a pressure relief valve on a basement water heater. Paneling in the basement and paneling were removed from the lower level by a mold remediator. The question was whether there was cross-contamination.

Discussion: Disturbed sampling resulted in a 7.5-fold increase in
Aspergillus-Penicillium spores compared to quiet sampling. The concentration increased from 260 S/m³ under quiet conditions to 1,950 S/m³ under disturbed conditions.

**Conclusion:** Disturbed sampling improved the detection of contaminant spores in a Condition 2 area and indicated some cross contamination had occurred (Chart 3).

**Example 4:** A basement water heater failed. Some wet building materials were removed during the initial response. There was no visible mold growth (VMG) reported and there was no moldy odor indicating active mold growth. The question was whether further mold remediation was warranted.

**Discussion:** Quiet mold sampling (Chart 4) revealed somewhat elevated total spores at about 5,000 S/m³ with moisture/leak indicator Aspergillus-Penicillium (ASP-Pen) spores comprising about 40 percent of the mix at 2,100 S/m³. Following fan disturbance, the measured total spore levels rose to well above the reliable upper limit of reporting of 100,000 S/m³ with virtually all now being Aspergillus-Penicillium spores.

**Conclusion:** The dramatic increase in Aspergillus-Penicillium spores revealed under fan-disturbed conditions revealed this to be a Condition 2 situation. Professional mold remediation was recommended (Chart 4).

**Example 5:** A second floor apartment in a 19th century building developed extensive mold growth on the exterior walls and window frame during the tenants second fall-winter of occupancy. A three-year-old resident became ill and developed asthmatic conditions. The previous summer an aquarium shop had opened on the opposite side of the ground floor (not under the apartment). After the parents wiped the walls and cleaned, the question was whether the apartment was suitable for reoccupancy.

**Discussion:** The paired data collected for total spores appear in Chart 5. Quiet sampling found 7,100 S/m³, of which 4,900 S/m³ were Aspergillus-Penicillium. Disturbed sampling found 1.5 million S/m³ of Aspergillus-Penicillium, a 300-fold increase. (Note: The author acknowledges that this figure is well in excess of what is accepted as the reliable upper limit of reporting: 100,000 S/m³. Nonetheless, it is the number reported by a very reputable lab.)

In addition to our investigation, about six days later, with no intervening remediation activity, an experienced mycologist was called on to carry out an investigation in the same apartment using a quiet sampling approach, impacting on an agar plate and then incubating. The mycologist did sampling for viable spores and found:

- Master bedroom: 380 CFU/m³ consisting of:
  - Penicillium at 300 CFU/m³
  - Cladosporium at 69 CFU/m³
  - Aspergillus glaucus at 11 CFU/m³

Those CFU data appear in Chart 6 with the vertical scale expanded 1,500-fold.

**Conclusion:** The disturbed sampling detected more than 300 times the amount of Aspergillus-Penicillium as quiet sampling, which also showed elevated spore counts. The later spore sampling significantly underestimated the degree of contamination. Professional mold remediation was recommended.

**Cleanup of Condition 2 Settled Spores**
Settled spores have eluded the traditional carpet fan and AFU/air scrubber approaches to remediation as can be seen in the above case studies. This is because mold spores are solid objects and cannot “jump” into the air on their own. They need drafts; but not any draft will do.

If you are trying to remove leaves from a driveway, you sweep the leaf blower back and forth across the surface and gradually corral/move the leaves into the pile you want. You know that if you just set up the leaf blower in the driveway and walk away, you will move the leaves off the surface only in the direct path of the exhaust while leaves on either side would just lay there even though...
the leaf blower’s blast is very strong. Similarly, a carpet/snail fan that is blowing in one direction without being redirected can mobilize only those spores in its direct draft, not those to the side, or above, or in the room behind it.

An alternative approach to reduce settled spore levels is to follow an “air polishing” protocol after the traditional approach of HEPA vacuuming and wiping down following the general guidance found in Section 12, Chapter 11 of the S520-2008. (Note: the process of air polishing is different than that of “air washing” that has been used in the industry for some time. Air washing usually involves just extended operation of air filtration units [AFU], commonly called air scrubbers.)

**Air Polishing Methodology**

The air polishing protocol involves maintaining the containment with workers still wearing protective gear, then:

- Ensure that AFUs being used are cleaned and evaluated.
- Depending on the size of the room and the AFU, place at least one AFU in recirculation mode in each area being remediated.
- Set up your room fans (oscillating type, similar to those described in the paired-sampling discussion). Approximate the number of fans to the size of the space. The smallest spaces (e.g., a bathroom or closet of less than 100 ft²) get one fan. For larger spaces, one to two fans per 100 ft², depending on the shape of the area.

- Oscillating fans should be positioned to maximize air disturbance in the quiet areas where spores can settle at the edges/corners of a room and on exposed rafters and joists. The goal

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**Pair Sampling Results Before and After Air Polishing**

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<th>Spores/structures/m³</th>
<th>After Initial Remediation</th>
<th>After “Air Polishing”</th>
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<tr>
<td>Disturbed B</td>
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</tr>
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<td>Quiet D</td>
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<tr>
<td>Disturbed D</td>
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**M Bedroom CFU Levels - Quiet**

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<th>M Bedroom CFU</th>
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**Chart 6 with vertical scale magnified**

**Chart 7 which includes data from Chart 1**
Air Scrubbers and Dehumidifiers are Not Alike

A common topic of discussion among mold remediationists is how long to operate air filtration units (air scrubbers) after HEPA vacuuming. This issue was raised during a discussion that indicated that boxy air scrubbers and boxy dehumidifiers are similar devices operating under the same principles. They aren’t and they don’t.

Air scrubbers are enclosures with powerful fans that draw air through a coarse pre-filter and then a large HEPA filter at rates of from 150 CFM to 2,000 CFM. HEPA filters remove airborne particles with a diameter of 0.3 micron with a minimum efficiency of 99.97%; and are expected to efficiently remove airborne fungal spores.

Dehumidifiers also move air but at slightly lower airflow rates of up to 400 CFM. In the air pathway are chilled metal fins that are kept below the dew point temperature of typical room air. When the room air contacts these fins, moisture condenses out of the air and is pumped to a suitable drain achieving water removal rates of from 150 pints to 300 pints per day.

While these devices have a similar appearance and move air with powerful fans, they have different operating principles:

- Air scrubbers remove airborne particles by trapping them in HEPA filters.
- Dehumidifiers remove water vapor from the air by condensing it out against refrigerated fins.

The difference is significant: A room can be dried with a combination of dehumidifier(s) and circulating fan(s) because the liquid water retained by the wet materials will evaporate faster. Once airborne, the water vapor can be removed by dehumidification, which increases the rate of evaporation and decreases drying time. Conversely, particles and spores are solids and have no vapor pressure. Once settled, they are not subject to removal by air filtration.

How Long Does It Take Spores to Settle Out?
Mold spores are in the size range of about 2 – 100 μ (microns)1. Particles and spores settle out of the air over time. The settling rate varies with spore size and air turbulence. In calm air conditions, spores near 3 μ will settle five feet in approximately 1.5 hours, while 100 μ spores will settle in less than six seconds2.

In light of this, operating an air scrubber for many hours to a couple of days in calm air conditions will become increasingly ineffective, as most of the particles that were initially suspended have either been scrubbed out of the air or settled to surfaces in the room well before the first day is over. Longer operation of air scrubbers alone will not improve the removal of the spores. Settled spores must be resuspended in order to be scrubbed from the environment.


Cleanup Case Study

Chart 7 shows the results from air polishing alone, with no additional HEPA vacuuming, on the project described in Example 1 of the sampling case studies.

Recall that the failure of two baseboard heaters led to damage from direct water as well as condensation. Four areas in the house (A–D) were contained and remediated by HEPA vacuuming and wiping down. Post-remediation paired sampling revealed elevated spore levels in areas B and D.

In area B the total spores were measured at 1,222 S/m3 using the quiet sampling method. However, the total spores increased 18-fold when the disturbed air sampling method was used, and within those samples, the Aspergillus-Penicillium (Asp-Pen) spores increased 24-fold, indicating that substantial numbers of settled spores persisted after traditional remediation had been done. In area D the difference was even more dramatic. The quiet sampling, at 944 S/m3, again seems acceptable. However, the total spore levels increased 41-fold when measured using the disturbed air sampling method, with Aspergillus-Penicillium spores increasing 120-fold.

Discussion: As Chart 7 illustrates, in Area B, the quiet spore levels were...
removal. The disturbed spore levels were reduced from 21,289 to 956 s/m³, a reduction of more than 95 percent.

In Area D, the effect of air polishing was even more dramatic. The quiet spore levels were reduced from 944 to 112 s/m³, an 88 percent reduction, and the disturbed spore levels were reduced from 39,056 to 600 s/m³, a 98.5 percent reduction. Aspergillus-Penicillium spores were reduced from 322 to 56 s/m³ in quiet sampling, an 83 percent reduction. And the disturbed sampling spore levels were reduced from 38,767 to 244 s/m³, a reduction of 99.4 percent.

Conclusion: The air polishing technique was able to dramatically reduce the level of total spores in areas with Condition 2 contamination without additional HEPA vacuuming.

Summary
With the release of IICRC-S520 there was recognition that Condition 2 settled spores (cross-contamination) needed to be addressed and remediated along with Condition 3 mold contamination. The question was how to determine if Condition 2 was present when the spores involved were not visible. These case studies demonstrate the value of paired (quiet/fan-disturbed) sampling to show when settled spores are present either before or after remediation.

The disturbed sampling results are significantly greater than the quiet sampling results, often by more than a factor of ten. The disturbed sampling integrates spore conditions by mobilizing spores into the air for collection in spore traps and comparing to an initial, quiet sample. Fewer samples need to be collected to characterize an area than would be required by tape/wipe sampling which, to avoid subjective sampling, requires scores of samples per area.

The results from paired sampling inform the remediator whether an initial effort, or additional effort, is needed to ensure a satisfactory end result.

Using the paired sampling approach as a model, an “air polishing” process can be utilized to improve on the overall remediation effort after normal HEPA-filter cleaning of surfaces. The use of strong leaf blowers at the air polishing stage — not at the initial, preremediation, baseline investigation stage — aims at aggressively moving any settled spores into the air. Several oscillating fans minimize the quiet zones and keep the spores airborne longer for filtration by the air filtration units (AFUs). After a few rounds of relocating the exhaust from AFUs, oscillating fans and additional leaf blowing, it can be expected that far fewer settled spores will be left over, resulting in a high likelihood of a successful PRV evaluation and fewer return trips that extend the remediation. This will save money for the client and shorten the overall remediation while achieving documented success — a cleaner space.

Sources and Notes
1. IICRC S520-2008 Standard and Reference Guide for Professional Mold Remediation, Section 3 (page 17)

>> ABOUT THE AUTHOR

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