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NAVIGATING THE NEW COLORADO ROOFING REPAIR LAW, PART 2

What Makes a Leader?

Why Cheating a Little Hurts a Lot

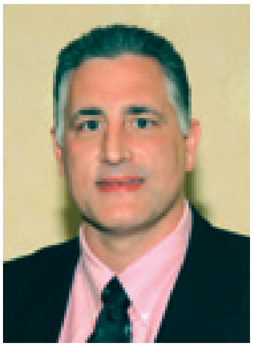
The Secret to Accurate Mold Sample Analysis

5 Questions With Damon Gersh

By Michael Pinto, CSP, CMP

The Secret of Mold Sample Analysis

Magnification Makes the Difference



The analysis of air and surface samples is a critical part of any mold investigation or remediation project. Nevertheless, many practitioners do not realize that analysis of spore trap samples currently is more of an art than a science. The scientific procedures of the analysis process are not very rigid, even for the most basic aspects of the work. With little strict guidance in the industry, the variation in sample results makes it seem as if each lab is offering its own perspective on the data. Much like multiple artists painting the same sunset, the results can be pretty startling when viewed side by side.

Although this variance between sample results has been a topic of discussion for many years in analytical circles, the concerns about the accuracy of sample analysis gained traction in the wider restoration industry when Larry Robertson made a presentation at the Indoor Air Quality Association conference in Las Vegas. His presentation, which graphically pointed out a large variation by various laboratories in both the type and number of spores identified on a single sample, mimicked work done previously by other professionals in the field.

Basics of Spore Trap Analysis

To accurately identify spores using light microscopy (i.e., using a regular microscope like those used in school biology classes), the analyst must consider size, shape, texture, septation,[1] attachment scars and color of the objects seen through the lens. With all these characteristics to observe and interpret, it takes time to evaluate some spores, especially smaller types. During the analytical process, the microscopist needs to focus up and down on a particular field of view on the slide and use higher magnification to determine some of these characteristics.

While large fungal spore types are relatively easy to identify and count, small spore types are very difficult and time-consuming to quantify, particularly when there are heavy concentrations on the slide. Misidentification of small spores can occur if all six characteristics are not taken into consideration, leading to erroneous conclusions. For example, smaller round types of *Cladosporium*, ascospores and basidiospores can be misidentified as *Aspergillus/Penicillium*-like spores (or vice versa) if an analyst fails to carefully observe distinguishing characteristics due to time constraints or the use of too low a magnification.

Speed of Analysis Versus Detail

A number of reports have confirmed that many laboratories typically allow a microscopist six to eight minutes to analyze a bioaerosol sample slide. In contrast, for counting fibers of asbestos, which is much simpler, the National Institute of Occupational Safety and Health (NIOSH) specifies five to 25 minutes for counting each slide.[2] To accommodate this "need for speed," laboratories choose to analyze for bioaerosols at low magnifications (150–400X). However, since important characteristics are not visible at lower magnifications, analysts can misidentify bubbles or particulates as spores or vice versa.

Figures 1–4 illustrate these challenges. The same portion of a slide was photographed at various magnifications. As can be seen, a much larger area of the slide is in view at lower magnifications. However, the distinguishing characteristics of the fungal spores are much more definitive as the magnification increases. The differences between fungal spores and non-fungal material are also more evident.

Certification Programs to the Rescue?

A number of programs and voluntary standards have addressed these issues. Both the American Industrial Hygiene Association (AIHA) and the Pan American Aerobiology Association offer certification programs for the identification of fungal spores using light microscopy. AIHA's Environmental Microbiology Proficiency Analytical Testing (EMPAT) program is considered the gold standard in the industry; however, successful participation requires only lab proficiency,

FIGURE 1

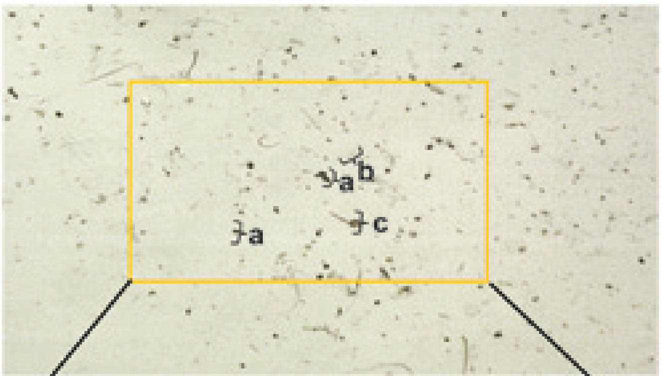


FIGURE 2

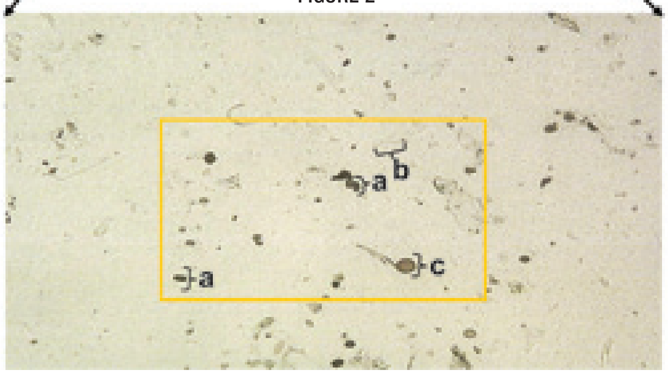


Figure 1
A bioaerosol sample magnified 100X. Within the area outlined in yellow, there are a) *Stachybotrys,* b) *Aspergillus/Penicillium*-like spores, and c) a smut/*Periconia*/ myxomycetes type spore. The overall deposition of material on this sample is low, yet the fungal spores and other particulate are, for the most part, very difficult to distinguish at this magnification.

Figure 2
The same sample magnified 200X. The image encompasses the area bordered by the yellow line in Figure 1. Each piece of particulate and fungal material is twice the size it was in the previous image. However, increasing the magnification decreased the area that can be observed at one time through the microscope. At this magnification, more detail can be seen. The smut/*Periconia*/myxomycetes type spore (c) can be observed easily. The three *Stachybotrys* (a) within the yellow border are more apparent, but at this magnification level they are still difficult to distinguish from other opaque material. The chain of *Aspergillus/Penicillium*-like spores (b) is faintly visible with careful focusing.

FIGURE 3

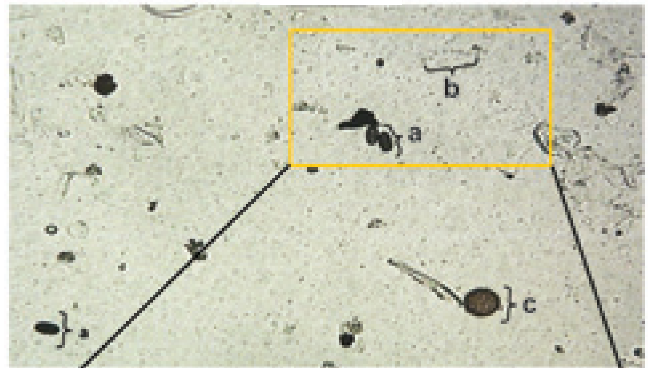


FIGURE 2

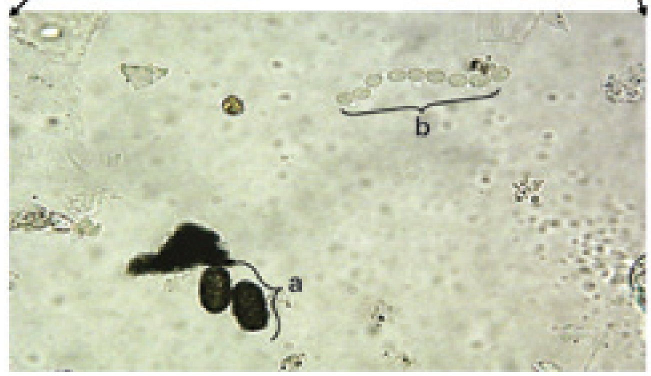


Figure 3
The bioaerosol sample magnified 400X. This image encompasses the area bordered by the yellow line in the image at 200X magnification in Figure 2. In this image, the materials on the sample have again doubled in size and even more details are apparent. The features of the smut/*Periconia*/myxomycetes-type spore (c) are very distinct. The *Stachybotrys* (a) are much more obvious and the chain of *Aspergillus/Penicillium*-like spores (b) is definitely visible, but still difficult to distinguish from background material and easily overlooked. Random single spores of this size would be even more difficult to distinguish from other round particles or bubbles on the slide at this magnification.

Figure 4
The same sample magnified 1,000X. This image encompasses the area bordered by the yellow line in Figure 3. In this image, the materials on the sample are 2.5 times larger than in Figure 3 and 10 times larger than in Figure 1. Fungal spores are easily distinguished from other particulate material on the sample. All the features of the *Stachybotrys* (a) and *Aspergillus/Penicillium*-like spores (b) are clearly visible and, thus, each type is easily identifiable at this magnification.

not individual analyst proficiency. PAAA offers individual certification for fungal spore analysis, but does not certify laboratories as AIHA does. Neither program addresses actual laboratory protocols used on real-world samples, such as time per slide, magnification utilized, percentage of trace observed, etc.

On May 1, 2009, the American Society of Testing and Materials (ASTM) approved a standard test method for categorizing and quantifying airborne fungal structures in a bioaerosol sample by optical microscopy.[3] For identification of larger spores, including *Stachybotrys*, *Memnoniella*, and *Chaetomium*, the standard allows a minimum total magnification of 150X. For identification of smaller spores, such as *Aspergillus/Penicillium*-like spores, *Cladosporium*, and smaller ascospores and basidiospores, the standard allows a minimum total magnification of 400X. While the standard states that a higher magnification may be necessary in certain situations (*e.g.*, high particulate levels, bubbles, particulate that looks like fungal material), it provides no guidelines.[4]

While this standard was long overdue and does provide minimal guidelines for laboratories to follow, it still

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allows labs considerable discretion in the exact standards utilized. As noted previously, a number of studies have shown significant variation in results among laboratories using protocols that would technically be acceptable according to the ASTM standard.

Multiple comparison studies have produced similar results. Because of different magnification utilized for microscopic counting and identification of fungal spores, the counts vary widely between labs. Consistently, these comparisons have shown that counts at 600X were approximately two times higher than at 400X magnification. Counts at 1,000X were approximately two times higher than at 600X magnification; this means that counts at 1,000X were approximately four times higher than at 400X magnification. Even to the layperson, this is a substantial variation that could radically alter the perception of the area that the sample represents.

Given that current evidence indicates that neither laboratory certification nor adherence to minimum standards is likely to result in consistent, high-quality results, a customer needs to consider more than price or certification when choosing a lab. Factors to consider should include a review of individual analysts' training, availability of an experienced microbiologist/analyst to assist more inexperienced analysts, proper monitoring of performance through internal and external quality control activities, secondary review of data, description of the type of microscope optics and magnification used on everyday samples, percent of trace analyzed, and time allowed for analysis.

This list of quality control measures may be unwieldy for non-laboratory professionals to apply to their sample analysis needs. But if customers do not start asking hard questions and demanding a better product from labs, the entire industry of mold identification and remediation will remain more art than science. RIA

Michael Pinto, CSP, CMP, is the CEO of Wonder Makers Environmental, Inc. He has more than 30 years of safety and environmental experience from jobs in the private sector, the non-profit arena and regulatory agencies. Pinto is the author of five textbooks and more than 150 published articles. He can be reached at map@wondermakers.com.

The author would like to acknowledge the assistance of Wonder Makers Laboratory Manager Joyce Cialdella, MA, for the help she provided in generating the images for this article and overall support of the material. She has more than 25 years' experience in pharmaceutical research working with soil microorganisms. For eight of those years she was also the curator of the corporate culture collection containing more than 12,000 organisms, including 4,000 fungal cultures.

Footnotes

1. Refers to a division of the fungal spore into distinct parts by a cell wall or septum. Many spore types have identifiable subsections and viewing the septation is key to proper identification of such materials.
2. NIOSH Analytical Method 7400, *Asbestos and other fibers by PCM*, item 19, Note 1 states: "A minimum counting time of 15 seconds per field is appropriate for accurate counting." Since item 18d of the method requires a minimum of 20 fields and a maximum of 100 fields be counted, a sample analysis time of five to 25 minutes is expected. Sample prep time must also be added to this estimate.
3. Representatives from Wonder Makers were participants in the ASTM process.
4. Many participants in the standard-making process argued that since all the characteristics necessary for definitive identification, even with high levels of debris, can be observed at 1,000X magnification, that level of magnification should be mandatory in those cases. However, the arguments for a more detailed analytical approach in which there is less likelihood of misidentification were defeated in favor of faster analysis.