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Determining Facility Mold Infection

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Consistent mold presence at high recovery levels indicates facility infection.

With the publicity surrounding the recovery of mold from pharmacy compounding facilities and formulated products connected to patient fungal infections and deaths, there has been a heightened awareness and concern for fungal recoveries in all manufacturing facilities. One comment often heard is that the presence of bacteria in a drug or biological manufacturing facility is expected, because these microorganisms are constituents of normal human flora, but the presence of mold in such facilities is presumed to be atypical as fungi are not considered constituents of human flora. Molds, however, are often found on healthy human skin and associated with hair. At any given point in time, humans may carry dermatophytic molds typically of the genera *Trichtophyton*, *Microsporum*, or *Epiderophyton*. Although mold are not present in or on humans in the numbers that bacteria are, they certainly can be found.

Humans cannot live in a world that contains at all times substantial concentrations of airborne mold and not be expected to carry some mold along with them as they move from point to point. Mold will be present on clothes, skin, and human hair as a direct consequence of the level of airborne mold that typical individuals inevitably encounter on a daily basis. Reasonable precautions upon moving to sections of facilities where products are aseptically manufactured should include changing into captive shoes and clothing and donning of hair and shoe covers. These precautions, however, cannot be reasonably expected to completely obviate the possibility of random, low-level mold recovery during routine monitoring. It is reasonable to expect that recovery rates for bacteria should be higher than for mold, but it is unreasonable to think that mold should never be recovered. An enforced zero recovery target level for mold is unreasonable, unscientific, and unnecessary.

Indoor air quality and mold

The presence of mold has become a key component in efforts to achieve appropriate indoor air quality in all environments inhabited by humans. Mold contamination in residential environments or a workplace can represent a significant health risk if conditions are such that mold infection of the building has occurred.

To this end, general classifications have been established regarding acceptable levels of mold that can be present in residential buildings. Baxter, et al. (1) suggests levels for two mold genera that are those most likely to produce respiratory allergies, which is the principle concern with airborne mold. *Aspergillus* and *Penicillium* are associated, in sensitive individuals, with allergic rhinitis, bronchial asthma, and alveolitis. Generally, total spore counts of 1200 spores/m^3 are considered safe or "clean", while counts above 1300 spores/m^3 are indicative of a "moldy" environment. Concentrations of *Aspergillus* and *Penicillium* below 750 spores/m^3 are considered generally clean, and counts above 900 spores/m^3 are considered moldy. Concentrations of ascospores or basidiospores below 1200 spores/m^3 are considered generally clean, and counts above 1300 spores/m^3 are considered moldy (1).

It should be noted that the devices and methods used to recover molds in residential and commercial buildings, included those with water damage, are a gross measure of the relative types and amount of mold in those environments. They are generally performed by environmental engineers, not microbiologists. The quantitation is typically performed using a compound light or phase contrast microscope physically counting a sample trace on a tape at 300x or 600x power. The identifications are performed based on "spore type."

The measure of airborne mold spores per unit volume determined by such methods, therefore, will not precisely correlate with recovery and growth on microbiological media sampled with a calibrated instrument, with results expressed as colony forming units (CFU). Generally, plating efficiency would be substantially less than mold spores/m^3 as the latter will reflect spores that are not viable or not recoverable on a given growth medium. It is well documented that mold spores are ubiquitous and those mold spores present at low levels in uncontrolled indoor environments pose an insubstantial risk to humans or products made in those environments.

Mold contamination risk in drug production

Fungi produce substances, in the form

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of secondary metabolites, which can be toxic to humans and animals. The concern in drug and biological manufacturing most often mentioned relates to aflatoxins and other mycotoxins, some of which may be tumorigenic in animals. Historically, these mycotoxins have found their way into animal feed and have had, at times, a devastating effect on the commercial poultry industry. There are concerns that mycotoxin related disease may be under-reported in human populations particularly in less developed regions of the world. There is little doubt that vigilance is required to ensure that mycotoxins do not present a risk in human and animal food and food ingredients.

react strongly to the recovery of mold at intermittent frequencies and at low levels.

Identifying, remediating, and preventing facility infection

To reach spore levels, to say nothing of toxin levels relevant as health risks, mold proliferation (i.e., growth) is necessary. This condition would require a production facility to be infected by mold. Conditions that allow a production facility to become infected are always objectionable. A reasonable question is: How would one diagnose the condition of facility infection?

Facility infection. A facility infection is indicated by the ongoing and consistent presence of mold. Given the microbiological assessments used in drug and biological production facilities, this means high viable recovery levels. There have been reports of building infection even in aseptic manufacturing areas, but fortunately these have occurred infrequently. Those that have been reported were due to water leaks from defective pipes or roofs that soiled wall materials made of sheetrock or drywall panels, which are an excellent food source for mold. The normal course of this type of facility infection is that high counts are recovered over a discrete period of time; the microbiological control team then recommends disinfection of the facility. Disinfection succeeds in a temporary diminution in frequency of recovery and count, but within typically 7–10 days a subsequent bloom is observed, manifested again by atypically frequent recoveries. Additional cycles of disinfection produce similar outcomes, with high counts again appearing within typically one to two weeks post disinfectant treatment. If a sheetrock wall is infected with mold, the only effective remediation is to demolish the walls and reconstruct them with a mold-inhibiting material, such as Plascore pharmaceutical cleanroom panels or fiberglass.

The recovery levels observed in residential and uncontrolled commercial building infection are unusually high and far exceed the total microbial recovery levels recommended in *United States Pharmacopeia* <1116> or *Eudralex Volume 4, Annex 1* for classified manufacturing environments (2, 3). Should such conditions be observed in controlled pharmaceutical facilities, an extensive diagnostic program and further steps, including mold remediation, may be necessary. In the event that remediation is necessary, the work that must be done is often extensive and may require considerable demolition and reconstruction. A mold remediation will be disruptive to operations, and may have impact upon manufactured product or work in progress.

Limited facility infection. Mold infections within manufacturing facilities and laboratories may be limited to equipment such as refrigerators or incubators. Again, these are not transient, low-level contamination recoveries, but instead are findings reflective of active mold growth. Fortunately, this is typically an easier condition to treat than widespread facility-level mold infection.

A removal of all materials from the equipment followed by careful cleaning and disinfection will, in most cases, eliminate the infection. Preventive actions include avoiding spillage of organic materials such as media or test materials and, where a spill does occur, cleaning effectively and at a suitable frequency. Also, to prevent mold infection, maintaining dry conditions is key, water spills should be removed quickly, and conditions that favor the formation of condensate should be avoided. In most cases, limited mold infection in support areas, such as laboratories, should not pose significant manufacturing risk, particularly when treated promptly and effectively.

An enforced zero recovery target level for mold is unreasonable and unnecessary.

Of interest to readers of this article, however, is the risk that accrues to human healthcare product manufacturing as a result of mycotoxin production. It has proven difficult to determine a level of mold spore inhalation or of mold in or on materials that can be associated with clear medical risk relating to mycotoxins. There is no medical or toxicological evidence that a low-level environmental mold recovery in a production environment would result in greater risk to a product or end-user than low-level bacterial contamination. Certainly, airborne mold found intermittently and at low frequency is not indicative of mold colonization. Low-level mold likely finds its way into facility environments as a passenger on personnel entering facilities as well as on materials coming into and warehoused within facilities.

It is reasonable to expect firms to take strong countermeasures to minimize the entry of mold into a facility. The ubiquity of mold in the external environment and the characteristically high levels of mold spores in air make it impossible to prevent mold entry into any indoor environment with absolute certainty. Thus, it is unreasonable that low-level mold recoveries should result in regulatory comment or enforcement. Also, there is no microbiological or medical reason to

Conclusion

The key risk factor regarding mold in drug and biological operations is proliferation-driven infection. Infection of facilities or equipment is easily distinguished from transient low-level contamination. Transient low-level contamination will occur infrequently and randomly. There will be no evidence of

high-count levels being reached and no indication of persistence. Given the ubiquity of mold in nature and the levels with which they are present in outdoor air or in office complexes, the recovery of an occasional mold does not merit any particular concern.

On the other hand, evidence of mold proliferation indicative of infection of facilities or equipment must be taken seriously and requires the prompt implementation of corrective and preventive actions. In such cases, the potential effect of mold contamination on product should be carefully assessed.

In the authors' experience, mold recoveries typically occur in industry at levels far lower than bacterial recovery. Certainly, microbiologists should keep a watchful eye on patterns to ensure that they have the means to take note of changes in pattern. It is important that significant microbial changes not be missed or ignored.

References

1. Baxter et al., *J. Occup. Environ. Hygiene* 2 (1) 8-18 (2005).
2. USP, General Chapter <1116>, Microbiological Control and Monitoring of Aseptic Processing Environments, (US Pharmacopeial Convention, Rockville, MD, 2012).
3. Eudralex, *Volume 4: EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use: Annex 1: Manufacture of Sterile Medicinal Products* (Eudralex, March 2009 revision). **PT**