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MOLD CONTAMINATION IN THE PHARMACEUTICAL INDUSTRY

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WHAT IS MOLD?

A recent survey of students close to high school graduation were questioned on "Whether Fungi are plants? Or "Whether Fungi are bacteria?" Fifteen students responded that fungi are plants. The remaining 150 students responded that fungi are bacteria. The teachers were astounded by how many thought that fungi are bacteria (Moore et al., 2011a).

Fungi are neither plants nor bacteria. They are eukaryotes and have the complex cell structures and abilities to make tissues and organs like higher organisms (Moore et al., 2011a).

Fungi, which include the organisms that are called yeasts and molds make a very significant impact on human existence, e.g., (Moore et al., 2011a):

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- The following would not be available without fungi: bread, alcohol, soft drinks, cheese, coffee, the statins, cholesterol-controlling drugs (statins) and some antibiotics.

- They aid in the digestion of grass eaten by cows.

- They make plant roots work more effectively helping to provide corn, oats, potatoes, lettuce, cabbage, peas, celery, herbs, spices, cotton, flax, timber, and the like. They even aid in the production of oxygen for us to breathe.

- The ability of fungi to secrete enzymes into their environment to digest nutrients externally is used in biotechnology to start biotech processes like cheese production, distressing of denim to make stone washed jeans, and the like.

- They produce a variety of compounds used to make medications, e.g., cyclosporins, statins, and agricultural fungicides.

Unfortunately, fungi are not always good for us. They can be responsible for diseases of crops, human infections, and can produce harmful chemicals like mycotoxins.

WHY DO I CARE ABOUT MOLD?

Several high profile recalls of products due to mold contamination occurred including the following (Cundell, 2016):

- Pharmaceutical tablet recalls in 2009 through 2011 due to the mold generated Tribromoanisole (TBA) taints from wooden pallets. Lumber treated with Tribromophenol (TBP) in South America was used in the Caribbean. The high humidity in Puerto Rico resulted in mold growth on the pallets. This in turn resulted in fungal methylation of TBP to the volatile, odorous taint TBA. Although users did not like the odor, it was not a toxicological concern.

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- Sanofi Pasteur's 2012 FDA Warning Letter for sterile product manufacturing facility in Toronto Canada due to mold contamination. This recall started with flooding that lead to water damage. Fungal colonies grew in the water-damaged building materials. Although mold contamination was found, complete investigations and corrective actions were not conducted.

- The New England Compounding Center (NECC) of Framingham, MA recall of three lots of steroid product (17,676 syringes) following a multistate outbreak of fungal meningitis caused by the mold *Exserohilium rostratum*. In 2012, Food and Drug Administration (FDA) inspected this compounding center and issued an FD-483. The environmental monitoring data showed numerous environmental monitoring excursions out of limits. This facility was not operating in a state of control and resulted in 753 fungal meningitis infections across 20 states.

There are several concerns with mold contamination in pharmaceutical manufacturing environments including pathogenicity, allergic reactions, mycotoxins and the invasiveness of mold (Hubka and Moldenhauer, 2015).

Pathogenicity

There are types of mold that can be pathogenic in and of themselves. National Health Techniques (Anonymous, 2015b) has identified many types of fungi as pathogenic (Hubka and Moldenhauer, 2015). When performing risk assessments and product impact assessments relative to mold contamination one should include an assessment of the medical impact of this mold on patients.

Mycotoxin production

Some species of mold are capable of producing toxins, called mycotoxins that can be harmful to humans. These mycotoxins can

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be some of the most toxic substances in existence. There are several different types of mycotoxins. The types include (Anonymous, 2015a; Hubka and Moldenhauer, 2015):

- Aflatoxins, which are produced by *Aspergillus* and include Aflatoxin B1, B2, G1, G2, M1 and M2.
- Ochratoxin which includes Ochratoxin A, B, and C.
- Trichothecene which is produced by *Stachybotrys* and includes Satratoxin-H, Vomitoxin and T-2 mycotoxins.
- Fumonisins which includes Fumonisin B1 and B2.
- Zearalenone.

It is very difficult to find a comprehensive identification of all the applicable mycotoxins. Table 1 identifies some of the mycotoxin producers. It is important to note that many different organisms have not been studied relative to the production of mycotoxins. As such, it is important to research individual isolates to determine if more updated data are available for that organism relative to the production of mycotoxins. It is also important to check more than one database, as different authors cite different sources of information.

Mycotoxins are not alive, so you can't "see them" on a culture plate. Irradicating mycotoxins involves the breaking down of the mycotoxin so that they are no longer dangerous to humans. Some of them are inactivated with 5% sodium hypochlorite, while others require high temperatures (260°C) for at least 10 minutes for inactivation. Ozone has been reported to inactivate most types of mycotoxins, however it requires a level of ozone that also has safety concerns for humans. HEPA air filters do not effectively remove mycotoxins (they are as small as 0.1 microns), although activated carbon filters are able to remove mycotoxins from the air. While mycotoxins can lose their toxicity over time, it may take several years to do this (Anonymous, 2015a).

Table 1 Toxin producing fungi (adapted from Moldenhauer, 2017a)

Fungal identification	Mycotoxin(s) produced
Acremonium species	Can produce trichotecene, which is toxic if ingested.
Alternaria alternate	Capable of producing tenuazonic acid and other toxic metabolites.
Aspergillus candidus	It produces the toxin patulin and citrinin.
Aspergillus clavatus	It produces the toxin petulin.
Aspergillus flavus	Some species produce aflatoxins.
Aspergillus nidulans	It can produce the mycotoxin sterigmatocystin.
Aspergillus oschraceus	It can produce a kidney toxin that can result in oschratoxicosis in humans (aka "Balkan Nephroropathy.")
Aspergillus parasiticus	Strains of this species can produce mycotoxins in the aflatoxins group.
Aspergillus species	Many species may produce mycotoxins, dependent upon the food source and the fungus.
Aspergillus terreus	This species can produce the toxins patulin and citrinin.
Aspergillus versicolor	It can product mycotoxins, e.g., sterigmatocystin and cyclopiaxionic acid.
Bipolaris species	This fungus can produce the mycotoxin sterigmatocystin.
Chaetonium species	Can produce a mycotoxin called Chaetoglobosin.
Eurotium species	The health effects and toxicity are closely related to that of the *Aspergillus* anamorph.
Fusarium solani	Can produce trichotecene toxins.
Fusarium species	Several species in this genus can produce potent trichlthecene toxins. Produce vomitoxin on grains.
Neosartorya species	The toxicity is closely related to the *Aspergillus* anamorphs.
Penicillium species	Some species can produce mycotoxins.
Scedosporium species	Two species of this organism cause health effects that can be fatal, *Scedosporium apiospermum* and *Scedosporium prolificans*. (Not clear is this is a result of a toxin or not.)
Stachybotrys species	Toxins produced include: macrocyclic trichotecenes, varracarin J, roridin E, Satratoxin F, G & H, Sporidesmin G, Trichoverrol, and Cyclosporins.

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There was an incident with contaminated maize in Kenya in 2004 where 125 people died from contamination from the aflatoxin mycotoxins. Many cases of pets dying from eating pet food with mycotoxins in it have been reported (Anonymous, 2015a).

Allergic reactions

Some individuals have an overly sensitive immune system that responds to specific types of mold. When the mold spores are inhaled, the body recognizes them as foreign objects and develops allergy-causing antibodies to be developed to fight off the foreign object. Following exposure, memory antibodies remain that can remember this foreign object and respond to future attacks by this foreign object. The immune system typically reacts to this response by release of substances like histamine. This in turn results in itchy, watery eyes, runny nose, sneezing and other allergy symptoms (Mayo Clinic Staff, 2015) (Hubka and Moldenhauer, 2015).

Some of the most common molds that cause allergic reactions include: *Alternaria*, *Aspergillus*, *Cladoporium* and *Penicillium* (Mayo Clinic Staff, 2015).

In the *Mayo Clinic Newsletter* A Mayo Clinic Study Implicates Fungus As Cause Of Chronic Sinusitis 1999 (Mayo, 1999), the researchers stated:

> "An estimated 37 million people in the United States suffer from chronic sinusitis, an inflammation of the membranes of the nose and sinus cavity. Its incidence has been increasing steadily over the last decade. Common symptoms are runny nose, nasal congestion, loss of smell and headaches" ...
>
> "Fungus allergy was thought to be involved in less than ten percent of cases," says Dr. Sherris. "Our studies indicate that, in fact, fungus is likely the cause of nearly all of these problems. And it is not an allergic reaction, but an immune reaction." (Hubka and Moldenhauer, 2015)

Invasiveness of mold

The structure of molds, with their spores, allows for the spores to spread rapidly and linger in the environment. Since the spores are not defense mechanisms (like bacterial spores) but rather reproductive agents, the spores can provide for more growth of mold in a facility. In addition to growth due to spores, scientists have identified fungal propagules, which are any material that is used for the purpose of propagating an organism to the next stage in their life cycle, such as by dispersal (Hubka and Moldenhauer, 2015).

A hypha (plural hyphae) is a long, branching filamentous structure of a fungus. In most fungi, hyphae are the main mode of vegetative growth, and are collectively called a mycelium. Hyphae, which are the stick like parts that grow out of mold are delicate and can break off even with a slight air current. These hyphal pieces are found to be very small and very viable and are referred to as propagules. Hyphal fragments or mycelia are components of fungal growth (similar to the roots and branches of a tree); it is common to find small hyphal fragments in outdoor air and in indoor dust. Propagules, which are units that can give rise to another organism (Moldenhauer, 2017b), are important due to their small size, which can easily pass through a high-efficiency particulate air (HEPA) filter pore (Gorney, 2002; Hubka and Moldenhauer, 2015).

CRITERIA FOR MOLD GROWTH

Mold requires four things for growth: available mold spores (or propagules), food, the right temperature and considerable moisture. Mold spores are ubiquitous – they are literally everywhere. Methods to reasonably and reliably eradicate them are not effective in the environment (not in a cleanroom). Attempting to eradicate mold by eliminating the presence of mold spores is deemed non-feasible (Anonymous, 2015a; Hubka and Moldenhauer, 2015).

Molds are not fussy eaters. As long as the other three requirements for growth are met, they can live on very little nutrient.

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Basically, they live off any carbon atoms (organic substances present). The oil remaining from your skin touching a surface can provide sufficient nutrients for the mold to grow if it is not properly removed during cleaning and disinfection. Even the soap used for cleaning can provide a nutrient for mold growth. Even items like wood, paper, and organic fibers can be used as nutrients (Anonymous, 2015a). Studies performed on PVC IV bags showed that mold could obtain sufficient nutrients to grow from the IV bags alone. Because of the fungi ability to utilize so many nutrients it can be very difficult to control mold by eliminating their sources of nutrients (Hubka and Moldenhauer, 2015).

One thing many people overlook as mold food is the microscopic pollen in the air. Pollen is a terrific mold nutrient, allowing mold to even grow on glass with the right moisture present (Hubka and Moldenhauer, 2015).

Molds tend to grow at a variety of temperatures, from cold storage to those temperatures that are quite warm. It is common to find out that temperatures close to freezing (e.g., in a refrigerator) are not sufficiently cold to inhibit mold growth. Many believe that temperatures must be in the room temperature range (20°C to 25°C) for mold growth. In reality, molds grow quite well at 30°C to 35°C also. They can cause disease in humans, who have temperatures around 37°C. In fact, temperatures much warmer, like those in the tropics, are also conducive to mold growth (Hubka and Moldenhauer, 2015).

The majority of molds require moisture and it can be necessary in sufficient quantities for growth. Typically mycologists refer to this moisture as water activity necessary for growth. The specific water activity level necessary is different for different species of mold. Most molds have levels that correspond to corresponding relative humidity of at least 70%. Most major mold outbreaks occur where porous, cellulose-type materials have been kept wet by liquid water or sustained condensation (Anonymous, 2015a). Water leaks provide

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major opportunities for mold growth to occur (Hubka and Moldenhauer, 2015). Table 2 (from WHO, 2009) identifies the moisture levels required for growth of selected microorganisms in constuction, finishing and furnishing materials. In this document, the WHO organization has classified fungi based upon the water activity and relative humidity levels needed for the organisms to proliferate.

The mold categories are defined by WHO as:

- Primary colonizers, which can grow at a water activity less than or equal to 0.80.
- Secondary colonizers, which grow at a water activity level of 0.80–0.90.
- Tertiary colonizers, which require a water activity greater than 0.90 to germinate and start mycelial growth (WHO, 2009).

Product impact analysis

Thorough and complete investigations should be conducted when mold counts are out of limits. Sometimes, we tend to downplay the effect of higher counts and just release the product. It is important to understand how your product is used, and the likelihood for pathogenicity, allergenicity, mycotoxin production, and the invasiveness of the product. All of these considerations should be discussed in the impact assessment, prior to release of the affected batches.

MOLD DETECTION AND IDENTIFICATION

Mold detection

Mold is typically found in pharmaceutical processes as part of the environmental monitoring results, product bioburden testing results, and may be visible on surfaces, e.g., due to water leaks or incomplete housekeeping.

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Table 2 Moisture levels required for growth of selected micro-organisms in construction, finishing and furnishing materials (WHO, 2009)

Moisture level	Category of microorganism
High (a_w >0.90; ERH > 90%)	Tertiary colonizers (Hydrophilic) *Alternaria alternate* *Aspergillus fumigatus* *Epicoccum spp.* *Fusarium Moniliforme* *Muco plumbeus* *Phoma herbarum* *Phialophora spp.* *Rhizopus spp.* *Stachybotrys chartarum (S. atra)* *Trichoderma spp.* *Ulocladium consortiale* *Rhodotorula spp.* *Sporobolomyces spp.* Actinobacteria (or Actinomycetes)
Intermediate (a_w 0.80–0.90; ERH 80%–90%)	Secondary colonizers *Apergillus flavus* *Asperfillus versicolor* *Cladosporium cladosporioides* *Cladosporium herbarum* *Cladosporium sphaerospermum* *Mucor circinelloides* *Rhizopus oryzae*
Low (a_w <0.80; ERH < 80%)	Primary colonizers (xerophilic) *Alternaria citri* *Aspergillus (Eurotium) amsterdami* *Aspergillus candidus* *Aspergillus (Eurotium) glaucus* *Aspergillus niger (aka Aspergillus brasiliensis)* *Apergillus penicilliodes* *Aspergillus (Eurotium) repens* *Aspergillus restrictus* *Aspergillus versicolor* *Paecilomyces variotii* *Penicillium aurantiogriseum* *Penicillium revicompactum* *Penicillium chrysogenum* *Penicillium commune* *Penicillium expansum* *Penicillium griseofulvum* *Wallernia sebi*

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Following the recalls due to fungal contamination mentioned earlier, many pharmaceutical industry professionals re-evaluated their positions regarding mitigation of fungal contamination risk and may have found the following (Cundell, 2016):

- Failure to provide sufficient attention to fungal isolation and trending during environmental monitoring.
- Lack of knowledge on whether disinfection efficacy studies adequately mitigated risk of fungal contamination (e.g., is it effective against fungal spores?).
- Not understanding the concerns associated with water leaks in the facility and their impact on fungal populations.
- Not having sufficient equipment and/or technique to identify fungal contaminants to genus and/or species.

Selective media

There are several selective media useful to recover fungal contaminants (Cundell, 2016). (See Table 3)

Incubation conditions

Incubation to recovery mold isolates is a subject with many different opinions. For many years, pharmaceutical companies have utilized two different media for sampling the environment. One medium was designated for the isolation of bacteria and another medium was chosen to selectively isolate fungi (yeast and molds). These media were typically incubated at 20°C–25°C for a specified number of days for fungi and at 30°C–35°C for a specified number of days for bacteria.

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Fungal Growth Media adapted from Cundell (2016)

Fungal media	Description	Where used/comments
Corn Meal Agar (CMA)	Media with high cellulose content	Recommended for the isolation of *Stachybotys chartarum*
Dichloran-Glycerol Agar 18 (DG18)	Medium containing 18% glycerol used for the recovery of xerohphilic yeast and mold	This medium is used for xerophilic fungal isolation
Malt Extract Agar (MEA)	Gold standard medium for isolation and speciation in mold-contaminated buildings	This medium is recommended for general fungal isolation for indoor air monitoring mold contaminated buildings. Studies conducted over 5 years showed higher mold counts compared to monitoring with TSA alone, when monitoring the exact same sites. However, it is not clear if the shorter incubation time and different temperature were the real issue.
Potato Dextrose Agar (SDA)	General purpose medium for the isolation of mold	General purpose media used for mold isolation
Sabouraud Dextrose Agar (SDA)	Medium for the isolation of dermatophytes. Recommended for Total Combined Yeast and Mold Counts in USP <61>	Widely used in pharmaceutical companies for mold isolation
Trypticase Soy Agar	Routine medium used by many pharmaceutical companies for environmental monitoring	Controlled Environmental Testing Association (CETA) recommended use of this single medium for monitoring in compounding pharmacies
V8 Agar/V9 Agar[1]	Developed in 1965 as part of a research project, V9 Agar was noted to induce early sporulation in environmentally isolated yeasts and molds when compared with peptone and sugar based formulas. The primary ingredients of the media are V8™ juice and potatoes, thus the name V9 Agar. The naturally low pH makes the medium inhibitory to most bacteria.	Recommended for *Stachybotrys* and *Chaetomium* species

1 Cundell (2016) identifies this medium as V8, but other sources identify this medium as V9

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In 1998, Marshall et al., published an article, *Comparative Mold and Yeast Recovery Analysis (The Effect of Differing Incubation Temperature Ranges and Growth Media)*, which evaluated the use of a single type of media for the collection of both bacteria and fungi and specified a set of incubation parameters that included the use of two temperature ranges. They found equivalent recovery for molds when incubated on TSA and incubated at 30°C–35°C, for eight of the nine molds. Moldenhauer (2017a) found that most mold recovery occurs quicker when incubated at 30°C–35°C.

Kielpinski et al. (2005) generated comparison data for sterility testing that indicated for a variety of samples. They found that an incubation temperature of 32°C provided improved detection times compared to the compendial sterility test procedure for all microorganisms.

USP <1116> *Microbiological Evaluation of Clean Rooms and Other Controlled Environments* (current version) was revised in 2012 and indicates that when using two-temperature incubation of environmental monitoring plates, one should first incubate at the higher temperature to ensure recovery of Gram-positive cocci, followed by incubation at the lower temperature. This chapter was subsequently updated to only be applicable to aseptic facilities.

The media used in typical environmental monitoring evaluations may or may not contain neutralizing agents, such as polysorbate 80 and/or lecithin, to improve the recovery of microorganisms from areas that may have been exposed to sanitizers or other antimicrobial substances.

Gebala and Sandle (2013) compared a variety of fungal recovery media and found the best recovery results using SDA.

Gordon et al. (2014) compared the different incubation conditions used for environmental monitoring and found that they had better results using two separate media TSA and SDA and two incubation conditions.

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Since there is no single consensus on this subject, it is useful and important for a company to have a validation protocol that shows the efficacy of its environmental monitoring recovery, incubation conditions and media used. Guidance on these types of studies is provided in Moldenhauer (2014).

Mold identification

When mold is detected, it is important to determine the identity of the mold found. Many companies use traditional morphology and microscopic evaluation as the only method of identification. The correct identification is dependent upon not only how it looks but the age of the culture and the method of incubation. It was not possible to utilize some of the automated systems for identification. As such, this led to many conflicting results, and improper identifications. It is not appropriate to only use morphology for identification in pharmaceutical microbiology laboratories.

If classical methods are used, it is important to utilize experienced mycological expertise (Cundell, 2016).

Today, many systems have the ability to detect mold auto-matically. This topic is discussed in detail in chapter 8 of this book. These methods may utilize proteonomic or genotypic methods.

The classification systems utilized in the 1950s and 1960s, did not properly deal with fungi protists and bacteria. Whittaker (1969) published a new classification scheme with five kingdoms: Animalia, Plantae, Fungi, Protista (eukaryotic microorganisms and a mixed grouping of protozoa and algae) and Monera (prokaryotic microorganisms, bacteria and archaea). This system was widely accepted in the 1970s. There is a fundamental difference in the four eukaryotic kingdoms and the prokaryotes primarily due to the higher organism traits of eukaryotes, e.g., nuclei, cytoskeletons, internal membranes, and mitotic/meiotic division cycles (Moore et al., 2011b).

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A later breakthrough by Carl Woese (1987) identified that all organisms contain small subunit rRNA (SSU rRNA). They were given this name (SSU rRNA) because they form a small subunit of a ribosome. Woese further believed that the SSU rRNA would be a perfect candidate for as the universal chronometer of all life (Moore et al., 2011b).

Woese studied the relationships between different SSU rRNA gene sequences from different organisms. This resulted in many questions about the common beliefs used in the relationships between organisms. Several considerations were made for the phylogenetic studies, e.g., (Moore et al., 2011b):

- They must be universally distributed across the group chosen for study.
- They must be functionally homologous.
- They change in sequence at a rate proportionate with the evolutionary distance to be measured (the broader the phylogenetic distance being measured, the slower must be the rate at which the sequence changes).

Table 4, from Moore et al. (2011b) describes the ribosomal rRNA sequences used in the identification of fungi.

The study of cladistics is a method that aims to reconstruct the genealogical descent of organisms through objective and repeatable analysis leading to a natural classification or phylogeny. It results in the tree-branching diagram (called a cladogram or phylogenetic tree) that shows the pattern of relationships between the organisms based on the characters used (Moore et al., 2011b).

It is extremely useful to have personnel at your site that are familiar with reading phylogenetic trees, who can interpret if you have similar or totally different sources of contamination.

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Table 4 Ribosomal rRNA sequences used to identify and classify fungi

rRNA molecule	Structural rRNA?	Transcribed?	Level of conservation	Taxa that can be distinguished
18S r RNA (Small subunit RNA)	Yes	Yes	Highly conserved domains interspersed with conserved domains	From domains to classes
5S rRNA	Yes	Yes	Conserved domains	Classes and orders
28S r RNA (large subunit RNA)	Yes	Yes	Conserved domains interspersed with variable domains	From phyla to species
Internally transcribed spacers (ITS) 1 and 2	No	Yes	Variable domains	Species and closely related genera
Intergenic spacer (IGS)	No	Yes	Highly variable domains	Strains and races

Matrix-Assisted Laser Desorption Ionization Time-of-Fight (Maldi - TOF) is a proteomic method of fungal identification that is available. This method is useful as it is very accurate, inexpensive, and rapid. It is a type of mass spectroscopy. Some companies utilize this type of identification system alone to perform the necessary identification.

If desired, this proteomic method may also be subjected to a genotypic identification, especially if there is a product failure involved. rRNA sequencing methods have been found to be more timely and accurate in fungal identifications (Cundell, 2016).

The data obtained, including the identification, should be maintained and trended to evaluate whether there are trends occurring in the facility.

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SETTING LIMITS FOR MOLD CONTAMINATION

Establishing the appropriate mold limits is based upon several different factors, one of which is the manufacturing process type (Moldenhauer, 2017b). There is an unfortunate trend among some companies to develop a zero tolerance for mold contamination. This is not scientifically sound or appropriate in most cases (Akers and Lindsay, 2014).

Aseptic processes

The FDA's Aseptic Processing Guidance (2004), USP <1116>, and Eudralex Volume 4 Annex 1 identify limits for classified manufacturing areas used for aseptic processing. The limits established for these areas are very low. Should mold be present as part of this count, it may be necessary to perform an investigation into the source and remediation (Akers and Lindsay, 2014).

This is not to say that mold is desirable in these processes, it is not. Depending upon the source of the contamination, mold remediation may be difficult and expensive (Akers and Lindsay, 2014).

Non-sterile processes

Fortunately, there is compendial guidance in Pharm Europa 5.1.4 and USP <1111> and <1115> of acceptable mold counts for product bioburden. These limits can be useful in establishing limits for environmental monitoring, which currently does not have compendia limits. It is important to note that neither compendium expects a zero-tolerance for mold contamination!

Pharm Europa includes Tables 5.1.4.1 and 2 – Acceptance Criteria for microbiological quality of non-sterile dosage forms. These tables include a column entitled TYMC, i.e., total yeast and mold count (CFU/g or CFU/mL). In this column the allowable counts range for 10^1–10^4 depending upon the route of administration

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for the product. As such, in setting limits one must understand the route of administration for the affected products and set the limits based upon the tightest limits required. Within in text of the monograph, there is a definition of what the colony-forming units (CFU) limits are where (Pharm Europa, 2017):

- 10^1 CFU means a maximum acceptable count of 20.
- 10^2 CFU means a maximum acceptable count of 200.
- 10^3 CFU means a maximum acceptable count of 2000 and so forth.

The USP <1115> states: (USP, 2018)

> "Bioburden levels in the ranges of those recommended in *Microbiological Examination of Nonsterile Products: Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use* <1111> are recognized as safe and do not pose risk of infection or microbial toxins. Manufacturers should have a clear understanding of situations that could favor microbial growth within their facilities and materials and should implement practical countermeasures."

The USP <1115> has similar Tables 1 and 2 to the Pharm Europa. The range of allowable mold counts is from 10^1–10^3 depending upon the route of administration for the product. It also has a similar method of interpreting the maximum acceptable count for each CFU (USP, 2018) (Moldenhauer, 2017b).

When you have the acceptable limits that can be in your bioburden with information that that level of contamination is considered *safe*, you can conduct a risk assessment of how much impact the environment has on your product, e.g., is it a closed or open system; the inherent mold count in the product; and your company's comfort level of control to set a limit that is the same or lower than the compendial guidance. One may find it difficult to support limits higher than those in the compendia without supporting environmental and product data (Moldenhauer, 2017b).

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World Health Organization

The World Health Organization (WHO) has issued numerous guidelines relative to pharmaceutical products as well as to other aspects of life, including those on indoor air quality (WHO, 2000; 2009). For indoor air quality, the guidance indicates many health risks associated with exposure to molds and provides clinical evidence of health exposure to molds (see the referenced appendices for each document). While not specifying specific limits to be met, there are applicable requirements and guidance to establishing limits.

The WHO has issued indoor air quality guidelines including, "The right to healthy indoor air" and in Principle 6 it says:

> "Under the principle of accountability, all relevant organizations should establish explicit criteria for evaluating and assessing building air quality and its impact on the health of the population and on the environment." (WHO, 2000)

In this guidance, there are specific instructions as to actions to follow to ensure appropriate air quality inside of a building.

MOLD REMEDIATION METHODS

In accordance with the WHO (2009) guidance on indoor air quality "the presence of many biological agents in indoor environments is attributable to dampness and inadequate ventilation." Dampness is specifically attributed as contributing to both microbiological and chemical contamination in the area, which can result in health risks. As discussed earlier in this chapter, different mold types require different levels of humidity and water activity for growth. Some contaminants are a result of molds being brought into the area, called transient contaminants, and others are a result of the conditions within the area (see Table 2) called inherent contaminants. In Table 2 provided earlier there are differences between the primary, secondary and tertiary colonizers. As such, you can look at the

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organism identification and determine for inherent contamination the water levels present. This can aid in determining whether you have an "unknown" leak or other issue to be resolved.

Once mold is detected in the facility, the task of remediation begins. Many focus on the task thinking of mold spores as being the same as bacterial spores. Bacterial spores are single walled cells, usually formed in response to hardship of some kind, and allow for survival of the cell after the hardship is resolved. Mold spores are actually tiny reproductive structures. They are made to germinate and multiply.

Not all mold are equal and therefore remediation methods may be different based upon the mold identification. A three-log or more, reduction of mold may occur just with the use of 70% isopropyl alcohol. Other mold types are more resistant. They may require more stringent methods for remediation. There are many publications by Jim Polarine and Paul Lopilito on fungal contaminations that are easier and/or more difficult to remediate. (e.g., Barnett et al., 2007).

As such, it is useful to have disinfectant efficacy studies that show the effectiveness of your cleaning agents against the types of molds routinely recovered in your facility. Understanding the ability of the disinfectant to work for you is key, when routinely using disinfectants to remediate mold.

When you have visible mold on a wall or equipment surface, the concentration of mold present is likely very high. As such, the typical two or three-log reduction seen in a disinfection study may not be sufficient to eradicate the amount of mold present.

Some consultants indicate the need for 3X or 9X cleaning regimens to remediate a contaminated environment. These may or may not be effective, depending upon the disinfectants used, how they are used, the level of contamination present and so forth. The best remediation is prevention of mold contamination before it occurs!

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Contaminated building facilities

When you have problems in cleanrooms or testing areas due to seasonal or environmental molds, solutions like the use of ozone gas, chlorine dioxide gas, or nitrogen dioxide gas may be useful in decontaminating the facility. These methods can be effective in killing the mold and mold spores present, however if you continue to expose the environment to contaminated environmental air the area will quickly re-contaminate. As such, it may be necessary to routinely use this type of decontaminant until the "seasonal" contamination issue has passed. Some companies routinely implement these programs at a specified frequency in specified seasons. For example, a company may utilize monthly decontamination cycles from spring until fall.

Water damage due to leaks or other sources should be immediately addressed. Part of the corrective action should include an assessment of the risk of mold contamination and preventative measures should be utilized to reduce this risk.

There are various guidelines available on the internet to describe methods of mold remediation (Anonymous, 2017a).

High levels of mold in the facility and/or presence of specific harmful molds may require use of a certified technician to remove the mold and certify that the facility is safe for use by humans.

Contaminated equipment and/or surfaces

Similar to facilities, one can choose to utilize a cleaning and disinfection method to reduce contamination. Since many cleaning and disinfecting agents can have an adverse effect on the equipment surfaces, it may be useful to consider newer non-traditional methods, e.g., the use of ozonated water to decontaminate.

The gaseous options provided for building decontamination can also be useful for transient mold contaminants. It is only

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effective for inherent contamination, if the gas can penetrate the area where the mold is present. Note: It is also important to fix the conditions that allowed for inherent mold to be present.

Contaminated product

In most cases, remediation of the product may not be possible unless your regulatory submission provides for some type of product reprocessing. Key considerations in responding to the contamination include a root cause analysis of where the contamination came from, corrective actions to eliminate the source of contamination, and preventative actions to prevent recurrence.

After that, the issues arise in determining whether the product is safe for use or should be rejected. A product impact analysis should be conducted, keeping in mind the various health risks associated with mold as well as Good Manufacturing Practice (GMP) and quality requirements.

MOLD PREVENTION METHODS

The best way to deal with mold is to ensure it is *not* a contaminant in your process! It is like that old adage, "an ounce of prevention is worth a pound of cure."

There are several different technologies available to prevent the likelihood of contamination starting with facility design. Appropriate engineering controls should be used in the design to prevent leaking, water or condensation build-up, and the like. Where there is the potential for standing water or wetness, there is the potential for mold.

When doing reconstruction or repair procedures, it is important to realize that outside air has the potential for area contamination and mold spores. As such, appropriate safeguards should be put into place to prevent this air from coming into contact with

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production areas. It is also important to respond quickly to the need for repairs for water damage in the facility.

A good defense for building materials is to use antimicrobial products for mold prevention during the construction process. Facilities that utilize antimicrobial flooring product protection, antimicrobial paint product technology, and stain-resistant bathroom fixtures can help prevent the growth of mold and mildew. These types of concerns can be important as building materials often contain starches, organic adhesives and cellulose sugars, which are an ideal nutrient sources for mold (Anonymous, 2017b).

An example of the antimicrobial product is manufactured by Microban®. Some of the products for prevention include: mold inhibiting window coverings, odor-resistant air filtration systems, antimicrobial flooring, sink and sealant products and the like (Anonymous, 2017b).

There are antimicrobial (and antifungal) paints available that can be used in locker rooms, change rooms and other painted areas in the facility. Many of these products are based upon the use of silver nanoparticles. The types of areas where counts are typically high may have no counts or deeply reduced counts following application of the antimicrobial paints.

Case study of environmental mold isolation in a controlled manufacturing facility (Falco, 2017)

- *Problem statement*
  Atypical levels of mold recovered from an environmental monitoring test session in a Grade D environment.

- *Root cause*
  Potential source identified outside the classified area in an access room to the decontamination autoclave had recovery of same mold.

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- *Corrective actions*
  Cracks in the walls in the access room where mold was found were sealed and repaired, then cleaned and disinfected.

- *Additional preventive measure*
  MoldGuardian™, a mold prevention solution was applied to this adjacent, non-classified area walls and floors as a long term preventive action.

- *Verification testing*
  Additional routine air viable sample location added to the environmental monitoring sample plan.

- *Effectiveness check on corrective actions and preventive measures*
  Atypical levels or molds have not been recovered in the manufacturing facility post changes.

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Brian G. Hubka graduated from the University of Notre Dame and has worked as an Independent Manufacturer's Representative representing companies to sell products to mass merchandising retail such as Home Depot, Costco, Sears, Walgreens, Safeway and worldwide hotel chains. From 2004–2007 he worked at Nature BioSystems, Inc. as Director of Marketing and Sales, assisted in product development with German scientists specializing in Quantum Physics. Traveled to Germany three times including organizing 10 day exhibition and booth at World's Largest Automobile, IAA Show in Hanover Germany in September 2006. Helped to develop products to increase fuel economy and many health related products.

In 2008, Hubka started to work with Vladimir Podlipskiy taking an all natural ingredient mold remediation product and developing a Food Grade product for use on fungus attacking growing crops throughout the world and developing a Pharmaceutical Grade product for use combating fungal infections in humans.

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Jeanne Moldenhauer, Excellent Pharma Consulting, has more than 25 years experience in the pharmaceutical industry. She chairs the Environmental Monitoring/Microbiology Interest Group of PDA, serves on the Scientific Advisory Board of PDA, founded the Rapid Microbiology User's Group™, and is a member of ASQ and RAPS. She is the author of *Steam Sterilization: A Practitioner's Guide*; *Laboratory Validation: A Practitioner's Guide*; *Environmental Monitoring: A Comprehensive Handbook*; *Systems Based Inspections for Pharmaceutical Manufacturers: Rapid Sterility Testing* (with Margarita Gomez); *Preparing for a Regulatory Inspection — Aseptic Processes and Non-Sterile Processes*; and numerous other publications.