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COMMENTARY

Mold Control and Detection in Biological Drug Substance Manufacturing Facilities: An Industry Perspective

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Introduction

The purpose of this paper is to provide guidance and drive consistency in regards to mold monitoring in biologics processes and facilities. These recommendations, from the members of the BioPhorum Operations Group (BPOG) Bioburden Working Group, are intended to assist biopharmaceutical manufacturers develop mold control and mold monitoring strategies. Each manufacturer is unique; therefore, alternative strategies may be applicable and/or qualified.

Scope

This paper focuses on mold detection and control in biologics facilities (process/environmental), including specific guidance on mold levels and responses to mold isolation events. In this paper, the term *mold* applies to all fungal species (i.e., molds and yeasts).

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Background

The biopharmaceutical industry produces *non-sterile and/or low-bioburden* intermediates and bulk biologics—that is, drug substances (DSs)—using bioburden controlled processes in accordance to Q7A and Annex 2. Bioburden control in biopharmaceutical processes is mostly based on key systems and associated components that are common to most biopharmaceutical manufacturers as follows:

1) Environmental control

- Heating, ventilation, and air conditioning (HVAC) design and qualification
- Utilities (water systems, clean steam, gases) design, qualification, and control
- Environmental monitoring (EM) program

- Pest control
- Facility flows (equipment, materials, product, and personnel)
- Facility cleaning and disinfection
- Disinfectant efficacy studies

2) Personnel control

- Gowning
- Training

3) Equipment control and maintenance

- CIP/COP (clean-in-place/clean-out-of-place)
- SIP (steam-in-place)
- Piping and equipment design and qualification
- Preventive and corrective maintenance program

4) Process control

- Bioburden monitoring strategy and control, including sample handling strategy
- Raw materials control
- Process qualification
- Filtration strategy

All the abovementioned elements of the microbial control strategy are intended to reduce the risk of microbial contamination in the manufacturing process and, therefore, potential risk to product quality. These microbial control systems represent microbial hurdles that limit the quantity and types of microbial populations in a biologics facility manufacturing environment. Bacteria represent, by far, the most common type of microorganisms that can be isolated in a biologics manufacturing environment. Therefore, most microbial control systems are targeted to minimize bacterial populations, which are the key indicators of contamination risks, but mold species are a normal part of the environment. While the frequency of observation of mold species should be much lower in the highly controlled areas of the manufacturing facility, low numbers of mold are not unexpected events in non-aseptic processing environments because mold species can also be part of the normal microbial population of a biologics manufacturing facility. From an industry perspective, there is data and consensus that the frequency rate of mold isolation in the manufacturing environment and manufacturing process is much lower than the frequency rate of bacterial events, but it is also acknowledged that molds are present in a biologics manufacturing environment. In many cases, single mold isolation events have received a high level of scrutiny; the goal of this paper is to challenge this paradigm and provide the rationale for an enhanced control approach that focuses on trending of mold species as microbial indicators rather than on single isolation events.

Mold Monitoring in Biologics DS Facilities

The environmental and process monitoring program should be capable of the detection of mold species. These monitoring practices may be summarized as follows:

Process and Support Area Environmental Monitoring (EM)

The EM program is designed to detect aerobic microorganisms, including yeast and mold species. Low-level molds are not atypical in a manufacturing biologics facility, and there is supporting evidence that, for most mold species (1), monitoring systems intended for general EM control will also be effective for mold monitoring.

- Most mold species that can be found in a biologics facility environment can be detected by routine incubation on tryptic soybean casein digest (TSA) media (1). TSA is the general purpose media used for EM in biologics facilities. Most BPOG companies perform single incubation at 30–35 °C for at least 3 days. Use of TSA and associated dual incubation conditions might be, in some specific cases, applied for the detection of mold species if required by facility data.
- Manufacturers should have data to demonstrate that representative mold species (selected ATCC species and in-house isolates) are detected on TSA at the intended temperature/time incubation conditions defined for environmental samples (2). Adequate environmental detection is supported through

growth promotion of incoming media using representative yeast and mold species. Inclusion of in-house isolates from the processing environment provides further assurance of recovery.

- Incubation times/temperatures and the mechanism for evaluating viable results should be performed in a manner that maximizes colony resolution and prevents overgrowth as much as possible.

- Mold species isolated in EM samples that exceed facility-specific air/surface viable action levels should be identified to the species level (or at a minimum, to the Genus level), whenever possible. DNA sequencing is a reliable technique for mold species identification, which can be combined with other phenotypic and genotypic technologies.

- Unless otherwise justified by specific facility data, the general principles defined for routine air viable monitoring (sampling locations, frequency, and testing regime defined for bacteria) are also valid for mold monitoring in biologics facilities.

- Based on previous statements, specialized or selective monitoring of processing environments for mold flora in well-controlled process environments is not considered necessary on a routine basis.

Only in cases where use of TSA has proven to be ineffective for the monitoring of mold species (which will be associated to specific facility data or conditions), enhanced or selective media (e.g., Sabouraud dextrose agar, SDA) may be applied to detect mold species in the environment, using a modified monitoring program (potential drivers might be evidence of mold growth in facility areas by visual inspection, mold isolation in product samples, mold isolation in external testing, product complaints, and others). Such supplementary monitoring is considered exceptional and may be substantiated and documented on the basis of process- or facility-specific risk assessment/investigations. Examples of such a program include the introduction of periodic monitoring using selective media incubated at 20–25 °C on a defined frequency (e.g., quarterly, annually) to detect and identify to species level molds that are isolated in the facility.

Molds are more likely to be detected within the outer perimeter or boundary of the manufacturing areas, interstitial spaces, and also in coldrooms. These are areas with lower environmental classification or boundary airlocks, and they are designed to facilitate material and personnel transition points from warehouse and other areas. Analysis of routine monitoring data at these locations can provide a more systematic evaluation of the effectiveness of material controls (e.g., sanitization) applied at key material and personnel transition points and help to mitigate the risk of mold ingress to the more critical areas.

Molds can be key microbial indicators of changing conditions in the microbial control status of manufacturing facilities and, therefore, emphasis should be placed on mold trending programs, rather than on individual responses to single mold isolation events. Increased mold isolation in a facility needs to be investigated accordingly.

Mold trending criteria to be considered in the environmental trending programs may include:

1) Location/area where mold has been isolated.

2) Number of mold genera and species identified.

3) Relative ratio of molds to other microbial isolates in each classified area in the trending period. Shifts in mold ratios from one trending period to another require further investigation.

4) Comparative analysis with historical facility data.

Periodic review (as defined in facility trending programs) of mold trends in the EM program should be sufficient to verify that the status of current facility and detection controls is adequate.

Process Monitoring

DS manufacturing processes include process and environmental controls to minimize the potential for microbiological contamination, and analytical measures to monitor process intermediates for evidence of microbiological contamination.

Incoming raw materials and unfiltered media solutions might contain molds depending on the manufacturing process. However, based on process and equipment controls, molds should not be routinely isolated from product samples at any process stage, and they will be investigated if this is the case.

In an analogous manner to the processing environment, there is supporting evidence that, for most mold

genera, methods intended for general bioburden testing will also be effective for process mold monitoring (most BPOG companies use TSA at 30–35 °C for at least 3 days). The following considerations need to be taken for mold monitoring in product samples:

- Analysis for the presence of mold species is typically performed using a qualified bioburden method (typically membrane filtration) or validated rapid microbial methods.
- Typically, a defined sample volume is applied for membrane filtration or direct inoculation analyses.
- Manufacturers should have data to demonstrate that representative mold species are detected using the applicable bioburden assay at the intended temperature/time incubation conditions defined for process samples. Qualification of bioburden assay should also address sample dilutions if required.
- Incoming growth promotion of test media is required to ensure the validity of these detection controls, and should include relevant in-house isolates.
- Testing should be performed within a suitably controlled laboratory environment to minimize the potential for laboratory-introduced contamination (biological safety cabinet or laminar air flow hood).
- Bench microbiologists need to be trained to differentiate between different microbial isolates to ensure proper assessment of results to support timely responses within manufacturing, if necessary.
- Because detection is atypical, mold isolates in product samples (product-containing matrixes) should be identified to species level (or at a minimum, to Genus level), whenever possible. As stated before, DNA sequencing is a reliable technique for mold species identification, and it can be combined with other phenotypic and genotypic technologies.

Mold Control Levels

The mold isolation frequency rate in well-controlled manufacturing environments and processes is much lower than the frequency rate for bacterial events. Given that the number of molds is likely to be at low levels and less than established total microbial action levels/specifications, specific mold/yeast/fungi numerical criteria do not need to be defined. From an industry perspective, bioburden alert and action levels for EM and process samples are typically inclusive of molds. When these numerical criteria are statistically derived from actual results, such control levels are considered adequate. Only in those cases where EM, product testing, and mold trending results show specific elements of concern and potential impact on product quality, it should be assessed whether or not establishing specific mold levels would provide additional value.

A possibly more suitable mold control criterion includes an assessment of the frequency and distribution of low-level mold in routine trend analyses for other environment and process samples. Such analyses are likely to be more effective when reviewed on the basis of location or proximity to a manufacturing process, and they may also help to identify low-level trends to help prevent a future excursion. Different trending approaches may be considered. Some examples for defining an adverse trend are detailed as follows (these are just examples; companies might use different approaches as part of their routine microbial trending program):

- No more than three consecutive increases in the number of mold species or genera identified in a facility per trending period (increase in absolute number reported for three consecutive trending reports).
- Increased frequency (e.g., >20%) of isolation of low-level mold in processing environment in a trending period versus historical data.
- Statistically-significant increase of mold species (versus other microbial groups) in a trending period versus historical data (e.g., in quarterly or monthly reports).

In all cases, the monitoring program should specify which actions (e.g., investigation and/or preventive measures) need to be taken in case of an adverse trend.

Responding to Mold Isolation Events

As defined in the previous section of this paper (Mold Control Levels), specific mold/yeast/fungi numerical criteria do not need to be defined, and bioburden alert

and action levels for EM and process samples are typically inclusive of molds.

Mold isolation events below alert levels for EM and/or product bioburden monitoring may not require specific actions or full investigations, given limited risk of these events and if a good trending program is in place. Discovery of mold in manufacturing areas (depending on location), should lead to additional/special cleaning that is effective for removal of spores to prevent the spread of molds in other areas.

Mold isolation events exceeding bioburden control levels will require an investigation with the rigor commensurate with the risk (e.g., action level vs alert level excursion), and should follow the same principles defined for bioburden or EM investigations.

As indicated in the previous section of this paper (Mold Control Levels), it is expected that manufacturers will implement a formal microbial trending program in DS facilities that will include specific elements for mold trending of process and EM data. This program will include quantitative/qualitative assessment elements to identify an adverse trend in mold results. In the event that a potential mold adverse trend is confirmed based on these criteria, manufacturers should conduct a full quality investigation to confirm such adverse trend, identify the root cause, assess product quality impact, and define corrective actions to restore baseline values in the process/facility. Molds can be indicators of changing conditions in the microbial control of the process and manufacturing areas and, therefore, trend monitoring of molds provides more value than responses to single mold isolation events. When adverse trends are noted in mold monitoring data, the investigation may include, where applicable, the following elements:

- Obtaining feedback from a cross-functional investigational team (e.g., manufacturing, quality control, quality assurance, engineering, etc.).
- Performing laboratory and manufacturing investigations. Manufacturing investigation should include at least a review of operational changes in materials/personnel flows, gowning procedures, cleaning procedures, storage, and sanitization procedures for materials entering the manufacturing areas and new materials introduced in the facility.
- Investigating potential increased engineering/maintenance activities in the affected area that could have been associated with the adverse trend, such as construction activities.
- Investigating architectural and physical integrity (leaks, water intrusion, humidity) of the manufacturing area where the adverse trend has been noted.
- Investigating housekeeping practices (e.g., unnecessary storage of materials in classified areas, residual water or moisture after cleaning activities).
- Investigating changes in utilities systems/HVAC design and operation (temperature, humidity differential pressure and air change rate control setpoints).
- Investigating seasonal impact and/or severe weather conditions.
- Assessing potential product quality impact considering system/process affected, species identification, and literature references (3), as well as additional monitoring/historical data.
- Determining root cause or, at a minimum, most likely root cause.
- Implementing corrective and preventive actions (CAPAs), which might include enhanced monitoring plans for a defined period of time.
- Performing CAPA effectiveness check, where appropriate.

NOTE: EM with adverse mold trends does not necessarily indicate that product quality has been compromised but *does* indicate the need to investigate. A risk assessment should be performed to evaluate the extent of the impact to the process/facility and whether a process should be halted pending resolution of the issue and completion of a return-to-service plan.

Mold Prevention Practices

Molds are ubiquitous in nature and, therefore, are part of normal microbial flora in biotech DS manufacturing facilities. Microbial control in these facilities is based on several design and operational criteria that prevent and minimize risks of microbial contamination in the process stream and, therefore, risk to product quality and patients. Most of the standard microbial control

elements in a DS manufacturing facility (e.g., HVAC) are particularly effective against molds when compared to effectiveness against bacterial species, which highlights the value of mold control as a key indicator of potential breaches of some of these design/operational principles. The following topics should be specially considered when developing a facility mold control strategy:

- Cleaning and sanitisation: In many cases, molds/mold spores are more resistant than vegetative bacteria to cleaning regimes. Therefore, disinfectants used in facility cleaning need to be confirmed to be effective against representative mold isolates. Inclusion of an effective fungicidal/sporicidal agent in the routine disinfectant rotation is recommended. The effectiveness of disinfectant agents might need to be also verified for low temperatures, if applicable.

- Gowning: Humans entering the manufacturing areas can be one source of molds in these areas. Therefore, the gowning program needs to make use of comprehensive reviews to minimize ingress of molds associated with personnel flows (special attention required for shoe changes or use of shoe covers). Special consideration needs to be taken in situations where there is increased flow of people to manufacturing areas due to maintenance or engineering activities, which can sometimes be correlated with increased mold excursions.

- Material flows: Because mold spores can survive on equipment, the surfaces of incoming material and equipment (including cart trolley wheels) should be decontaminated (according to a proceduralized method) on entry to a processing area to limit microbial (including mold) ingress. Materials like wood, any kind of cardboard, and other cellulosic materials have to be kept outside of cleanrooms because they are difficult to adequately decontaminate.

- Coldroom controls: Many raw materials and process materials may be stored in coldrooms prior to processing. Coldrooms (including air vents and fans) should be cleaned and sanitized on a defined frequency to limit the potential for the proliferation of molds. Outer packaging may be removed and/or surface sanitization of materials should be performed on entry to cleanrooms.

- Facility flows: Material/personnel flows in the facility suites need to be designed to maximize unidirectional flows as much as possible.

- Invasive maintenance Procedures: Special consideration needs to be taken in case of situations where engineering projects are being executed in manufacturing areas concurrently with manufacturing activities in other suites. Complete isolation (hard and not soft, e.g., plastic, physical barriers where possible) and segregation of material and personnel flows need to be in place to minimize ingress of molds into manufacturing areas. Effective disinfection and confirmation of control should be applied prior to reinstating the area for process use.

- Raw materials: Same as described for personnel, raw materials and raw material containers can be potential sources of molds in manufacturing areas. Adequate vendor qualification and incoming raw materials control, as well as validated decontamination (disinfectant/contact time), need to be in place to minimize the risk of mold ingress from raw materials into the manufacturing area.

- Utilities: Use of sterile-filtered process gases and validated installation and operation of process water systems are very effective to prevent mold contamination linked to these utilities systems.

- HVAC design and operation: Proper design of HVAC systems (including appropriate grade and location of terminal filtration), as well as validated operating conditions (air changes/hour, temperature, and humidity in the suites and a preventive maintenance program) are also key elements to minimize mold isolation events coming from outside air.

- Facility events: Process events such as liquid spills should be remediated as quickly as possible.

- Overall facility design: It is widely known that architectural breaches/deficiencies are one of the root causes of mold isolation into manufacturing areas coming from interstitial spaces, black areas, areas behind equipment panels, or the outside environment. The manufacturing suites need to be properly designed and maintained to ensure physical segregation and to prevent contamination from these spaces (special attention to ceilings, walls, floors, and drains). Thus, preventive maintenance

and proper housekeeping in the manufacturing area represent an essential part of mold contamination prevention.

Conclusion

This paper provides recommendations from a biopharmaceutical industry perspective on mold monitoring in biologics DS facilities and processes. Additionally, recommendations on subjects commonly encountered in the establishment of a monitoring program, such as setting control levels, responding to mold isolation events, and best practices on mold prevention, are included.

These recommendations assist biologic manufacturers in refining their current mold control strategy, as well as developing control strategies for new processes, facilities, and products. Establishing appropriate mold control programs is a key element of overall microbial control plans in biologics manufacturing facilities. New facilities need to collect baseline mold data during initial facility qualification to ensure adequate mold assessments with future data. For existing facilities, actual mold data needs to be compared to historical data to identify potential areas of concern.

In the future, the BPOG Bioburden Working Group will use this paper to present an industry perspective on mold monitoring of biologic manufacturing processes to regulatory agencies to provide input regarding future regulations for non-sterile bulk biologic manufacturing.

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Since its inception in 2004, the BioPhorum has become the open and trusted environment where senior leaders of the biopharma industry come together to openly share and discuss the emerging trends and challenges facing their industry. BioPhorum currently comprises more than 1200 active participants in six forums—Drug Substance, The Development Group, Fill Finish, The Technology Roadmap, BioPhorum IT Group, and BioPhorum Supply Partners.

More information can be found at www.biophorum.com

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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