Microbiological Control for Affinity Capture Chromatography Processing: An Industry Perspective

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COMMENTARY

Microbiological Control for Affinity Capture Chromatography Processing: An Industry Perspective

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ABSTRACT: The purpose of this paper is to provide a summary of a BPOG-led industry survey of the microbiological control aspects of affinity chromatography processing in the biopharmaceutical industry. The document provides a summary of historical microbiological control concerns, coupled with industry-derived best practices, for material, equipment, and storage controls required to mitigate the potential for microbial ingress and contamination of chromatography resin and equipment. These best practice guidelines, which are derived from the members of the BPOG Bioburden Working Group, are intended to assist biopharmaceutical manufacturers to enhance microbial control and monitoring strategies for chromatography systems.

Introduction

Affinity capture chromatography is routinely applied for the purification of biopharmaceutical products. Affinity chromatography separates proteins on the basis of specific interaction between the product and ligand within the chromatographic resin. This separation technique is typically included in the initial stages of product purification in the downstream manufacturing process, and it ensures effective clearance of process and product-related impurities while retaining the product for further processing.

The design and operation of affinity capture chromatography in biopharmaceutical facilities include specific measures to ensure microbiological control. Because resin may be reused over multiple batches, and in many cases over long-term campaigns for a given product, storage controls are important to mitigate the potential for microbial colonization and proliferation. While these procedures also apply to many other resin types used in biopharma, affinity chromatography resins, due to the ligand proteinaceous nature, are usually

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incompatible with high temperatures or prolonged contact with microbiocidal solutions. In addition, the material presented for purification is frequently nutrient-rich, cell-free media from cell culture, providing potential opportunities for microbial proliferation.

The BPOG industry-led survey was performed to provide a historical assessment of microbiological control issues and to recommend control measures that may be implemented to provide effective microbial control.

**Purpose of Industry Survey**

The purpose of this survey was to develop best practices for microbiological control of the affinity capture chromatography unit operation:

- To outline historical microbiological concerns for this unit operation (i.e., source of microbial events, type of isolates recovered, etc.).

- Determine likely sources of microbial contamination.

- To focus on the material, environmental, equipment, and storage controls required to mitigate microbial ingress and biofilm formation in chromatography equipment.

- To list the potential remediation measures that may be taken during a microbial event.

### TABLE I

**Survey Results for the Application of Affinity Capture Chromatography within Biopharma**

<table>
<thead>
<tr>
<th>Utilization of Affinity Capture Chromatography Processes in Biopharma</th>
<th>● All survey respondents use an affinity capture resin as a product purification step.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>● A majority of respondents, 11 of 14, apply affinity capture as the primary purification capture step.</td>
</tr>
<tr>
<td></td>
<td>● A vast majority (13/14 respondents) use standard, off-the-shelf resin types. Examples include MabSelect™ (Select, Xtra, SuRe), ProSep® (vA, Ultra Plus, Ultra-pre-packed).</td>
</tr>
<tr>
<td></td>
<td>● Specialized resins may be required in some cases.</td>
</tr>
<tr>
<td></td>
<td>● Choice of resin type is product-dependent.</td>
</tr>
<tr>
<td></td>
<td>● Mixture of traditional and continuous processing applications.</td>
</tr>
<tr>
<td>Process Applications</td>
<td>● Resin is product-dedicated.</td>
</tr>
<tr>
<td></td>
<td>● Sub-cycles may be employed to complete the purification of one batch.</td>
</tr>
<tr>
<td></td>
<td>● Resin may be used in short or long processing campaigns.</td>
</tr>
<tr>
<td></td>
<td>● Lifetime is typically limited by maximum number of load cycles.</td>
</tr>
<tr>
<td></td>
<td>● Resin may be stored between manufacturing campaigns (some exceptions applied).</td>
</tr>
</tbody>
</table>

### BPOG Survey Method and Participation

All companies surveyed are biopharmaceutical manufacturers of both clinical and commercial cell culture products and are located predominately throughout the U.S. and Europe. Up to 14 respondents from drug substance manufacturers participated. Participants included subject matter experts from manufacturing, microbiology, and quality assurance. The survey was performed in a blinded manner, coordinated by a representative from the BPOG organization. The summary results are reflected in this document. The survey was initiated with general questions relating to chromatography and application within the manufacturing platform. The survey focused on historical events relating to microbiological control, with emphasis on potential points of microbial ingress, remediation procedures, and prevention controls. The recommendations for best practice from industry are included, comprising material, equipment, and operational controls.

### Survey Results & Discussion

The application of affinity capture chromatography within the industry group is summarized in Table I.

Manufacturing experience has determined that bioburden control is a key component of affinity capture processing. Table II illustrates the primary concerns.
The detail of specific bioburden control concern was further elucidated by the participants in this survey. As outlined in Table III, water-borne species and typical environmental isolates are the predominant isolates detected. Detection of the contaminants was readily evident in the routine process monitoring applied to equilibration effluent and product pools, and less frequently from swab surface monitoring of chromatography equipment. The isolate types detected were closely correlated to the confirmed or putative point of ingress. Of particular interest was the recurring detection of *Stenotrophomonas maltophilia*. These isolates were detected across multiple facilities and companies, with no commonality other than resin type and, in some cases, the sanitization strategy. Equipment hardware design and soft part failure were common causes of concern; these may be mitigated through use of pre-packed columns. Weak and ineffective storage solutions lead to contamination when applied in situ or when in storage out-of-column (e.g., for longer periods between campaigns). It is also acknowledged that resin material is not free of microbial species, with typical incoming raw material specifications at $<100$ CFU/mL.

The response to each event was dependent on the scope and extent of contamination. The remediation efforts included extensive sanitizations, and also included column re-packing, equipment cleaning, and so forth. In some situations the resin material had to be discarded.

The experience of the survey participants enabled the collation of guidelines or best practices to ensure effective control of the resin material such that microbial ingress could be effectively prevented. These controls are summarized in the following section.

**Best Practices for Affinity Capture Resin Storage**

Resin is stored both in and out of column. While the majority of respondents unpack and store offline when not in use, three of the (14) respondents maintained a preference to store columns *in situ* between campaigns. Two respondents do not apply resin storage at all and discard resin at the end of the manufacturing campaign.

Where storage conditions apply, the majority of unpacked resin is stored in carboys at 2–8 °C. The same storage buffer is typically applied for *in situ* and out-of-column storage. Storage solutions such as 18–20% ethanol (buffered solutions may apply) and 1–2% benzylalcohol at 2–8 °C (pH 3.2 or 5.0) are applied, based on compatibility with the resin, vendor guidance, and facility requirements. It was noted that the concentration of the solution in storage can be dependent on the unpacking procedures, the containment provided during storage, and the storage duration. Sporeformers are well known to persist in 20% ethanol solutions, with some noted observations of microbial persistence in pH 5 benzyl alcohol. Of the 10 respondents, four provide microbiostasis data to support resin storage.

### TABLE II

Survey Results of Historical Bioburden Control Concerns in Biopharma

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is bioburden a concern for affinity capture processing?</td>
<td>Ten of the 12 respondents consider the affinity capture step to be susceptible to microbial ingress and/or colonization.</td>
</tr>
<tr>
<td>What are the inherent factors which drive this concern?</td>
<td>1. Potential presence of nutrients in the charge and buffer solutions to support proliferation.</td>
</tr>
<tr>
<td></td>
<td>2. Packing and unpacking are open operations.</td>
</tr>
<tr>
<td></td>
<td>3. Storage solutions with limited bactericidal efficacy.</td>
</tr>
<tr>
<td></td>
<td>4. Limited sanitization options for resin type.</td>
</tr>
<tr>
<td></td>
<td>5. Potential for niche spaces in columns which may not be exposed to sanitization (i.e., difficult-to-clean areas).</td>
</tr>
<tr>
<td></td>
<td>6. Difficult-to-eliminate potential microbial contamination following ingress.</td>
</tr>
<tr>
<td>Historical experience with bioburden during processing</td>
<td>Two of 12 respondents have not observed significant bioburden issues and/or special concerns compared to other chromatography steps.</td>
</tr>
<tr>
<td></td>
<td>All 12 respondents have experienced a bioburden issue historically. These vary from a low-level isolated event to a recurring event.</td>
</tr>
<tr>
<td>Microbial Species Detected (in order of frequency detected)</td>
<td>Point of Detection</td>
</tr>
<tr>
<td>-------------------------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>● <em>Stenotrophomonas maltophilia</em>; <em>Pseudomonas</em> spp.</td>
<td>Typically:</td>
</tr>
<tr>
<td>● <em>Bacillus subtilis</em>, <em>Bacillus cereus</em>, <em>Bacillus thuringiensis</em>, <em>Bacillus alcalophilus</em></td>
<td>● Within equilibration effluent streams</td>
</tr>
<tr>
<td>● <em>Bacillus cohnii</em>, <em>Bacillus pseudofirmus</em>, <em>Bacillus thuringiensis</em>, <em>Bacillus thuringiensis</em></td>
<td>● Pre-filter eluate (i.e., product before filtration/pool)</td>
</tr>
<tr>
<td>● <em>Paenibacillus glucanolyticus</em></td>
<td>● At the column load step</td>
</tr>
<tr>
<td>● <em>Ralstonia picketti</em></td>
<td>● From rinse samples/pre-use flushes</td>
</tr>
<tr>
<td>● <em>Achromobacter</em> spp.</td>
<td></td>
</tr>
<tr>
<td>● <em>Corynebacterium</em> spp.</td>
<td></td>
</tr>
<tr>
<td>● <em>Microbacterium</em> spp.</td>
<td></td>
</tr>
<tr>
<td>● <em>Enterococcus</em> species, Gram-negative non-<em>Enterobacteriaceae</em></td>
<td></td>
</tr>
<tr>
<td>● Gram-positive cocci</td>
<td></td>
</tr>
<tr>
<td>● Yeast species</td>
<td></td>
</tr>
<tr>
<td>Note <em>S. maltophilia</em> biofilm may harbor other microorganisms&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Proteobacteria such as *S. maltophilia* have been reported to form stable biofilms. However, most biofilms are heterogeneous in population and may provide the synergic conditions for the biofilm formation. While *S. maltophilia* may be the dominants species in a biofilm, other species may be present at lower levels.
Where resin storage applies, validation of the effectiveness of the storage conditions is a regulatory expectation. Concurrent resin lifetime studies include an evaluation of resin storage, which may apply both in situ between batches, and out-of-column storage for longer term storage. Microbiological data is typically requested to support maximum resin storage durations.

In almost all cases (10 of 12 respondents), the effectiveness of resin storage is verified each time, through in-process control testing of the resin storage solutions or slurries prior to further processing. These in-process controls may include one of the following:

- through a blank elution prior to further processing,
- directly on the resin slurry/test of slurried resin before column packing,
- routine bioburden testing after sanitization of each column packing,
- equilibration of the first cycle,
- sampling of post-storage flush solution.

The benefit of these in-process controls was not evident in all cases, however, as only two respondents detected an issue with the resin prior to use. Due to the nature of downstream operations, it is not always operationally feasible to wait on bioburden data from the packing operation before further processing. The application of rapid microbiological methods would provide substantial benefit in this manufacturing step.

Short-term storage also applies within the chromatography column between batches and between cycles of individual batches. Storage controls and solutions used for such short-term intervals varied greatly among the respondents. For example, resin is sanitized and stored in 20% ethanol between individual cycles. This strategy incorporates a parallel sanitization and storage of the gradient cart equipment (i.e., chromatography skid) used for processing. Another strategy involves a defined sanitization and storage frequency, based on the number of cycles/batch or the processing duration (sanitization and storage after every 24 h). In these circumstances, the resin is regenerated between cycles and maintained in equilibrated solution until a defined time has elapsed or until each cycle of the particular batch has been processed across the unit operation. Continuous processing ensures no storage between chromatography cycles. A recommended bioburden control measure is to ensure that neither the packed resin nor the gradient cart equipment is stored in equilibrated solution for longer than 24 h. Where required for chromatography, gradient carts are stored in ethanol or caustic solutions (≥0.5N NaOH where possible). These microbiological controls are verified as a component of commercial-scale process validation of the unit operation.

Best Practice for Resin Selection

The frequency, duration, and intensity of resin sanitization are linked to resin lifetime and supporting studies. When selecting an affinity capture resin for use in manufacturing, ensure that microbiological control elements are considered with equal weighting to process performance and lifetime claims. The resin type should be amenable to frequent sanitization with a robust sanitizer to prevent microbial events in manufacturing. For example, where continued use of resin over longer periods is anticipated, it may be advantageous to select a resin type that is amenable to treatment using more robust sanitization solutions (e.g., 0.1 N NaOH). Such resin should also provide for better recovery from a microbial event.

Given the industry concern and potential impact to microbiological control during critical processing steps, the industry survey strongly challenges resin vendors to implement effective control measures for *Pseudomonas* species and/or reduce the raw material specification below ≤100 CFU/mL. More stringent specifications for bioburden are required at this juncture, and biopharma should proactively engage with their resin suppliers to facilitate such process improvements. It is clear that resin manufacturers also consider microbial control as a key raw material quality attribute, given development initiatives by certain resin manufacturers, which, for example, may soon include the ability to provide resin that is suitable for autoclaving.

The application of continuous processing has led to significant developments in resin management, with single-use columns and resin applied.

Best Practices for Control of Process Inputs

A key control to ensure that the packed resin is effectively protected for microbial ingress is control of process inputs. Contaminated charge/load solutions...
was highlighted as a common concern in biopharma. The nutrient-rich, cell-free media may provide low-level bioburden, which can colonize the resin bed, making it potentially difficult to treat with routine sanitization solutions, given the niche spaces provided in a compressed resin bed. On-skid bioburden control filtration is applied as the primary measure of control for this reason. While guard filters may be used to protect the resin within the column, it is generally recommended that the load solution is subject to sterilizing-grade filtration prior to loading to the Protein A column. Filtration of incoming process and cleaning buffers may also be enabled in certain situations. Once process buffer connections have been established, avoid scenarios that require reattachment, as this increases the need for further manual interventions. These controls are accompanied by use of hygienic connections, aseptic technique, and effective training.

**Chromatography Equipment and Soft Parts Management**

Process equipment and system components should be designed to minimize the potential for microbial contamination. Because common water-borne isolates have a proclivity to form biofilm in difficult-to-clean areas, the equipment should be designed and qualified to ensure effective cleaning and removal of residue and excess water rinsate. Ensure line slopes are provided for maximum drainability. Difficult-to-clean locations (e.g., inlet ports and valves) should be thoroughly inspected to ensure effective cleaning. The equipment should be designed to minimize any open operations.

Gradient carts utilize a variety of fixed and disposable components and are generally not subject to steam-in-place prior to use. Particular emphasis should be provided to the design of efficacious cleaning regimens to mitigate the risk of biofilm formation on the equipment surfaces. The general guidelines for equipment cleaning and soft parts management are thoroughly described in PDA Technical Report No. 69—Bioburden and Biofilm Management in Pharmaceutical Manufacturing Operations (1). Contamination events have been described in which cleaning steps were not performed with sufficient flow rate to remove air, leading to ineffective application of the sanitizer. Effective cleaning is ensured when equipment flow path and flow rate applied for sanitization is optimised and validated for microbial control. Following cleaning, gradient cart design principles should allow for complete draining, which prevents standing water. This is especially important in situations where gradient carts are stored dry. Where gradient carts are stored in bacteriostatic solutions following cleaning, removal of excess cleaning rinsate is also important.

Cleaning validation of ancillary equipment is also important. Effective validation for cleaning of empty columns, slurry tanks, resin storage containers, and packing skids has been demonstrated to improve microbial control for affinity capture chromatography operations.

Robust preventative maintenance procedures are necessary for this equipment. Regular inspection of soft parts for damage are required, with a change-out frequency which is based on thorough risk assessment of process use, contact with sanitization solutions, steam, and so on. Because misalignment of gaskets and failed control valve diaphragms can lead to ineffective cleaning and biofilm formation, inspection of seals and gaskets for leaks is required, with pressure testing of column seals recommended prior to use. Use of double-sealed O-rings provides additional protection to the process flow paths. Specialized components such as chromatography column gaskets/frits used for resin support may be subject to re-use and offline storage. Specialized storage and return-to-service procedures should be developed for such components.

**Effective Resin Sanitization**

Following a satisfactory resin pack operation and closure of the column, resin sanitization is utilized as the primary measure to control bioburden. The sanitization solutions applied vary across the companies surveyed and are largely resin-dependent or recommended by resin vendors based on compatibility studies. Typical examples include low concentrations of NaOH in NaCl-buffered solution (typically for MabSelect); however, guanidine HCl followed by acetic acid/benzyl alcohol or acetic acid/ethanol are also used.

Sanitization is applied in almost all cases after column packing and prior to the first cycle of each batch to be processed; however, thereafter the frequency of sanitization varies based on resin type and application in manufacturing. At least five of nine respondents sanitize the affinity capture resin after each cycle has been processed. The remaining respondents apply a sanitization schedule in line with the start and end of the
processed batch (i.e., cycle number–dependent) or in a
time-dependent manner (e.g., at least once per day
during processing). The method of application varies,
with almost half of the respondents applying the san-
itization solution in up- and down-flow mode. This is
recommended particularly following an intervention
(e.g., column packing). The contact time with the
sanitization solution varies greatly (15–120 min) and
is largely applied without a static hold.

Each sanitization solution will have its advantages and
limitations. For example, it is well known that low
concentrations of NaOH are only effective for vege-
tative species, as Bacillus spp. have been observed to
persist without additional intervention. Based on his-
torical observations, many of the respondents have
developed remediation measures for a suspected
bioburden event.

The sanitization regimen is typically developed and
assessed initially through laboratory-scale evaluations.
However, the effectiveness of sanitization for micro-
bial control can only be verified through continuous
process verification at commercial scale. Based on
these considerations, general best practice guidelines
for resin sanitization include the following:

- The frequency of sanitization is important for mi-
crobial control. Column steps that do not have a
frequent cleaning tend to have many more micro-
bial events.

- The application of an effective regeneration (i.e.,
strip) step may enhance the effectiveness of sani-
tization step.

- For larger columns, ensure sufficient flow rate to
ensure radial diffusion (via the column flow plate)
throughout the column.

- For larger columns, ensuring homogenization of
the resin during the packing helps to prevent chan-
neling or other sources of uneven flow.

- Applying sanitization in up- and down-flow mode
is known to enhance dispersion of sanitizer within
compressed resin bed, while also helping to re-
move entrained air.

- A static hold allows for static diffusion of the
sanitant throughout the compressed resin bed.

- Develop remediation measures for a suspected
bioburden event (see below).

Effective Remediation Measures for a Suspected
Bioburden Event

Detection of bioburden in equilibration effluent or
product pools during successive chromatography cy-
cles is likely to indicate a microbiological trend that
requires effective corrective measures to restore con-

trol. While each event and the detected species will
vary in each situation, almost half of the respondents
have proactively determined remediation measures to
be deployed. Examples of such measures are outlined
in Table IV. The compatibility of the resin with stron-
ger solutions is an obvious prerequisite. To facilitate
such measures, a number of respondents indicate that
laboratory-scale lifetime studies incorporate such re-
mediation measures. While measures should be taken
to ensure a representative challenge, it is acknowl-
edged that these laboratory-scale studies cannot pre-
dict every manufacturing event over the lifetime of the
resin, and also do not have significant breaks between
every three to five cycles as required in traditional
manufacturing. Remediation at commercial scale
therefore needs to determine the steps necessary based
on systematic risk assessment to ensure consideration
of both the performance and lifetime of the resin and
the requirement to re-establish microbial control. Note
that in some cases, bioburden events are not easily
resolved using these methods. In some situations, resin
was unpacked and discarded.

Best Practices for Affinity Capture Packing and
Unpacking Operations

A majority (7 out of 11) of the respondents identified
column packing using fresh or re-used resin as the
processing operation that is more susceptible than
others to microbial ingress. Column packing is an
open operation, where the resin is slurried in a buffer
solution before being packed into a clean column.
Companies have developed procedures to minimize
these risks, with particular emphasis on limiting the
time required for packing and by applying a post-
packing sanitization activity. Additional recommenda-
tion is to provide for bacteriostatic solution storage for
the resin if there is a delay in packing or when packing
performance analyses need to be repeated.

The resin unpack process is also an open process.
Where resin is subject to storage, the decanting oper-
ation to storage containers may be required. The majority of respondents perform these activities using a manual or semi-automated process. A packing skid is usually required. The common procedures applied to enhance control of these activities are as follows:

- Perform column packing and unpacking activities under cleanroom controls. A minimum Grade D classified area should be provided for this open operation.

- Where possible, provide a dedicated area.

- Close up the unit operation as much as possible.

- Gowning controls should include full coverall and sterile gloves/gauntlets and face masks, to minimize risk of personnel contamination during open steps.

- Limit the number of personnel in the area during the operation. Ensure personnel have specialized training and are trained in good aseptic technique.

- Limit the time required to conduct activities.

- Where possible, pack the resin in process solutions that limit microbiological growth (e.g., use storage buffers). If this is not possible, limit the time the unpacked resin remains within solutions that permit microbial growth.

- Utilize autoclaved components where possible.

- Verify for bioburden in a rinse sample after empty column cleaning.

## Conclusion

A BPOG-led survey has been carried out with the purpose of establishing industry best practices for microbiological control considerations of affinity capture chromatography processing in the biopharmaceutical industry. All survey respondents indicated that affinity capture is used as a step in the product purification process at their facilities. The survey reveals that bioburden concerns during affinity capture processing remain prevalent. All (12 of 12) respondents have experienced varying degrees of bioburden issues ranging from low-level isolated events to adverse trends. Despite this experience, a vast majority (10 of 12) of the respondents still considers the affinity capture processing to be susceptible to microbial ingress and/or colonization. Reasons cited for the ongoing concerns include potential presence of growth-promoting incoming materials, open operations particularly during column packing and unpacking, use of weak storage solutions, limited availability of resin sanitization options, potential presence of hard-to-reach spaces within the column resulting in diminished effectiveness of cleaning solutions, and the inherent difficulty of eliminating potential future contamination following a microbial ingress event. The most commonly recovered organisms found in such events were *S. maltophilia* and *Pseudomonas* spp.

### TABLE IV

**Potential Remediation Measures That May Be Taken on Detection of a Suspect Bioburden Event**

<table>
<thead>
<tr>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>● Apply a corrective sanitization using a more robust sanitization solution. MabSelect SuRe: 0.1–0.5 M NaOH; Prosep Ultra: 3% benzylalcohol (with 0.3% HCl). Acidification of the benzyl alcohol significantly improves the microbial kill kinetics, enabling effective sanitization.</td>
</tr>
<tr>
<td>● Some companies perform proactive sanitization with a stronger solution following high-impact activities (e.g., after packing and prior to each batch).</td>
</tr>
<tr>
<td>● Repeated sanitizations (e.g., three consecutive) may be more effective than a stronger solution in some cases.</td>
</tr>
<tr>
<td>● Physical removal may be more important to remove sporeformers (higher caustic levels may not be effective in isolation).</td>
</tr>
<tr>
<td>● Increase the flush volume and sanitize in up- and down-flow mode.</td>
</tr>
<tr>
<td>● Empty columns can be passivated in response to detection of biofilm.</td>
</tr>
<tr>
<td>● Preventative maintenance procedures and soft part inspection frequency is enhanced.</td>
</tr>
<tr>
<td>● Manual cleaning of valves may be performed in difficult-to-clean locations.</td>
</tr>
<tr>
<td>● Separate sampling of gradient cart and column rinse water generally demonstrates the contamination location at column level.</td>
</tr>
</tbody>
</table>

- Verify for bioburden in a rinse sample after empty column cleaning.

**Conclusion**

A BPOG-led survey has been carried out with the purpose of establishing industry best practices for microbiological control considerations of affinity capture chromatography processing in the biopharmaceutical industry. All survey respondents indicated that affinity capture is used as a step in the product purification process at their facilities. The survey reveals that bioburden concerns during affinity capture processing remain prevalent. All (12 of 12) respondents have experienced varying degrees of bioburden issues ranging from low-level isolated events to adverse trends. Despite this experience, a vast majority (10 of 12) of the respondents still considers the affinity capture processing to be susceptible to microbial ingress and/or colonization. Reasons cited for the ongoing concerns include potential presence of growth-promoting incoming materials, open operations particularly during column packing and unpacking, use of weak storage solutions, limited availability of resin sanitization options, potential presence of hard-to-reach spaces within the column resulting in diminished effectiveness of cleaning solutions, and the inherent difficulty of eliminating potential future contamination following a microbial ingress event. The most commonly recovered organisms found in such events were *S. maltophilia* and *Pseudomonas* spp.
The survey results offer best practices in various areas of affinity capture chromatography processing, including control of process inputs, equipment design considerations, as well as resin selection, storage, and sanitization. Recommended best practices include performing column packing and unpacking activities in dedicated areas of the cleanrooms wherever possible, ensuring personnel complete good aseptic technique and any other applicable specialized training, utilizing autoclaved parts and process solutions that inhibit microbiological proliferation such as storage buffers, limiting the time for critical activities, and testing for bioburden after column cleaning. Potential remediation measures mentioned following a suspected bioburden event may employ the use of a more effective sanitization solution or performing repeated sanitizations, physical removal especially when spore-formers are recovered, increase in flush volume with a robust or high flow rate and sanitization via both up- and down-flow modes, passivation if biofilm is suspected, manual cleaning of valves or column preventative maintenance to replace all loose parts, and separating sampling of gradient cart and column rinse water to help determine the contamination location at the column. Resin reuse over multiple product batches is common within the biopharmaceutical industry, and microbial ingress and/or colonization can significantly reduce the number of times the resin can be reused. Therefore, adopting the best practices described in this survey may have a significant impact on the successful management of affinity capture chromatography processing.

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The authors wish to acknowledge Andrea Moran (Lilly), Nigel DePeiza (Astra Zeneca), Kris De Smet (Sanofi), William Schaut (Janssen), Barbara Daddis (Bristol-Myers Squibb), David Bain, and other members of the BioPhorum Operations Group (BPOG) Microbial Control workstream for their contributions to this article.

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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