Quality Risk Management of Microbial Burden in Parenteral API Production Processes

Herman Herz, Anne-Lise Bache, Birgitte Holst

This paper describes a risk assessment technique that may be used to control bioburden in parenteral active pharmaceutical ingredient (API) production processes. Readers can use the risk assessment technique to achieve bioburden control during API processing. The objective in creating this tool was to develop a way to establish a balanced control strategy regarding bioburden.

INTRODUCTION
Processing biotech active pharmaceutical ingredients (APIs) under aseptic conditions is not required by regulations. However, good manufacturing practice (GMP) regulations state that the control of microbiological contamination should be appropriate in relation to risk to the patient (1 §113, 2 §86, 3, and 4). Quality risk management (QRM) has been chosen as a method by Novo Nordisk for achieving this “appropriate” control, because as International Conference on Harmonisation (ICH) Q9 (5) states, “effective quality risk management approach can further ensure the high quality of the drug (medicinal) product to the patient by providing a proactive means to identify and control potential quality issues during development and manufacturing.” An overview of the development and manufacturing activities is shown in Figure 1.

The purpose of this article is to provide sufficient detail that readers can use the QRM tool presented herein to achieve bioburden control during API processing.

AUTHORITY EXPECTATIONS
Controlling the bioburden in API production is an increasing challenge to many manufacturers because of an increase in the complexity of biotechnology processes. The global guidelines (3, 4) provide manufacturers with limited guidance for controlling bioburden that includes the following:

- §4.32 If drinking (potable) water is insufficient to assure API quality, and tighter chemical and/or microbiological water quality specifications are called for, appropriate specifications for physical/chemical attributes, total microbial counts, objectionable organisms and/or endotoxins should be established.
- §5.23 Where equipment is assigned to continuous production or campaign production of successive batches of the same intermediate or API, equipment should be cleaned at appropriate intervals to prevent build-up and carry-over of contaminants (e.g., degradants or objectionable levels of micro-organisms).
- §11.13 If the API has a specification for microbiological purity, appropriate action limits for total microbial counts and objectionable organisms should be established and met. If the API has a specification for endotoxins, appropriate action limits should be established and met.
- §18.13 Depending on the source, method of preparation, and the intended use of the API or intermediate, control of bioburden...during manufacturing and monitoring of the process at appropriate stages may be necessary.
- §18.13 Specific for biotech products: The raw materials used (media, buffer components) may provide the potential for growth of microbiological contaminants.
Bioburden is defined as the level and type (e.g., objectionable or not) of micro-organisms that can be present in raw materials, API starting materials, intermediates or APIs. Bioburden should not be considered contamination unless the levels have been exceeded or defined objectionable organisms have been detected.

This paper helps to determine acceptable bioburden levels. The definition of objectionable organisms is considered facility, process, and product specific and is, therefore, outside the scope in this paper. The term “tool” in this paper refers to the described risk assessment technique to control bioburden in parenteral API production processes.

**DEVELOPMENT OF THE ASSESSMENT TECHNIQUE**

In searching for a way to control microbiological contamination when processing biotech APIs, the aim was to develop a tool that could establish a balanced control strategy regarding bioburden. The risk assessment technique scientifically predicts microbial contamination without testing and trending, as this would involve a massive development program. Initially inspiration came from references 6 and 7. By focusing on sources of microbiological contamination and factors in the process that can cause variability (Figure 2), a scoring system to prevent unacceptable levels of bioburden was developed.

Risk assessment techniques used within the pharmaceutical industry (4) often begin by identifying the hazards, defined as the potential source of harm (4). The relative risk to patient is hereafter determined by multiplying severity and probability of occurrence.

This QRM tool only uses the probability of occurrence to prioritize the effort and establish the control strategy for the critical quality attributes (CQA) bioburden and endotoxin. If the severity is applied, it is a constant that will not influence the outcome of the assessment when the tool is used.

When management are mitigating risk and accept residual risk, the severity of the risk to the patient should be considered. The severity should be individually applied per product. The value for severity depends on the drug product and is not covered in this paper.

Bioburden and endotoxin are critical quality attributes that are linked to the drug product. The final stages of preparing a parenteral drug product normally involve preparing the final formulation by mixing the API and excipients, sterile filtration,
The development of the risk assessment tool focused on how sources of microbiological contamination and factors in the process (Figure 2) contribute to the probability of occurrence of bioburden.

The sources and factors were grouped into the following seven variables:

A  Time
B  Temperature
C  Process Material
D  Packaging Material
E  Equipment Status
F  Interventions
G  Environment.

Equation 1 is used to generate a risk priority number (RPN) for each processing step. The mathematical equation reflects the seven variables, and the relationship between them, for contributing to microbial contamination, as follows:

\[
RPN = A \times B \times ([5 \times C] + D + [2 \times E] + [F \times G])
\]

[Equation 1]

The equation, which combines the seven variables and the relationship between them, was developed together with experienced subject matter experts (e.g., microbiologists, process technicians, and process development scientists) and with inspiration from publications, including references 6 and 7.

Initially, the equation was piloted at process steps for commercial products. Incidents from the past were used to challenge the robustness of the equation. These pilots provided the baseline and prepared the method to be used for new processes and changes in process designs.

The RPN was used as a measure for how balanced the control strategy in each process step was and lead to appropriate and prioritized actions when the identified RPN for a process step indicated that the number needed to be lowered (Table I).

The sources of microbial contamination and the factors in the process that can cause variability have different impact on the CQA of the API. Steps that precede the final endotoxin-removing step are considered low impact, due to the purifications steps that follow, while steps after the final endotoxin-removing step are considered high impact (Figure 3).

To distinguish between initiatives to be taken in high impact steps and low impact steps, two sets
of action limits for the RPN values can be applied (Table II).

**GENERATION THE RISK PRIORITY NUMBER**

Each variable is given a score from 0 to 2 (Table III) to characterize scenarios going from best-case scenario = 0 to worst-case scenario = 2.

Experience showed that assigning a score to characterize the scenario for process material can be challenging. Table IV was therefore developed at a later stage.

**PERFORMING THE BIOBURDEN RISK ASSESSMENT**

The principle steps of the bioburden risk assessment are shown in Figure 4. The relevant team members, including a microbiologist, start with a process flow diagram (Figure 5) of the main flow of production activities and processes feeding into the main flow (i.e., production of buffers, chromatography regeneration, etc.) This diagram can be used to do the following:
- Generate an overview
- Estimate how long the risk assessment process will take
- Define where the risk assessment should start and finish
- Allow agreement on what defines the process
- Identify all the process steps that need to be assessed; normally each shape with a light blue background (Figure 5) generates its own RPN.

Once the process step is mapped and each variable in a process step is assigned a score, a RPN can be calculated (see Equation 1 and Figure 6) for each step.

Worst-case situations should be used when assigning scores to the variables (i.e., if the temperature within a storage tank is normally around 10°C.

**Figure 3:**

The main production process and relative levels of risk from microbiological contamination.

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**TABLE I: Actions associated with RPN ranges.**

<table>
<thead>
<tr>
<th>RPN Range</th>
<th>Consequence</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>High RPN</td>
<td>Insufficient control strategy</td>
<td>= Reduce risk</td>
</tr>
<tr>
<td>Medium RPN</td>
<td>Improvements of the control strategy should be considered</td>
<td>= Reduce risk if possible</td>
</tr>
<tr>
<td>Low RPN</td>
<td>Sufficient control strategy</td>
<td>= Accept risk</td>
</tr>
</tbody>
</table>

**TABLE II: Applying action limits to low-impact process steps and high-impact process steps.**

<table>
<thead>
<tr>
<th>Process steps (see Figure 3)</th>
<th>Low-impact steps</th>
<th>High-impact steps</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent risk to product quality</td>
<td>Low-impact steps</td>
<td>High-impact steps</td>
<td>Action</td>
</tr>
<tr>
<td>High</td>
<td>Upper RPN range</td>
<td>Upper RPN range</td>
<td>Reduce risk</td>
</tr>
<tr>
<td>Medium</td>
<td>Medium RPN range</td>
<td>Medium RPN range</td>
<td>Reduce risk if possible</td>
</tr>
<tr>
<td>Low</td>
<td>Lower RPN range</td>
<td>Lower RPN range</td>
<td>Accept risk</td>
</tr>
</tbody>
</table>
### TABLE III: Assigning score to characterize the scenario for the variables (CFU = Colony Forming Units, CIP = Clean In Place, and SIP = Sterilization In Place).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Process Time</th>
<th>Process Temp 0°C (8)</th>
<th>Process material</th>
<th>Packaging material properties</th>
<th>Equipment properties</th>
<th>Interventions</th>
<th>Environment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
<td>G</td>
</tr>
<tr>
<td>0</td>
<td>&lt; 1 min.</td>
<td>&lt; 0 or &gt; 65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>&lt; 10 min.</td>
<td>0–8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&lt; 2 hours</td>
<td>8.1–17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>&lt; 10 hours</td>
<td>17–25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>&gt; 10 hours</td>
<td>25.1–65</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **Probability Factor**
- **Process Time**: Time required for the process
- **Process Temp 0°C (8)**: Temperature of the process
- **Process material**: Material used in the process
- **Packaging material properties**: Properties of the packaging material
- **Equipment properties**: Properties of the equipment
- **Interventions**: Interventions required during the process
- **Environment**: Environment conditions during the process

- **Score**: Score assigned based on the scenario described in Table III
- **Sanitary design**: Sanitary design of the equipment
- **CIP**: Clean In Place
- **SIP**: Sterilization In Place
- **Process integrity in control**: Process integrity maintained under control
- **Low content of CFU after cleaning and in control**: Low content of CFUs after cleaning
- **Manual cleaning/Manual assembling**: Manual cleaning or manual assembling
- **Design with elements that are not sanitary**: Design with non-sanitary elements
- **Automated process with manual interventions**: Automated process with manual interventions

### Notes

- **Temperature correction**: If the temperature is over 20°C due to seasonal variation [warm in the summer] then the temperature of over 20°C must be entered into the scoring scheme. This will give a scoring factor of 2 instead of 1 (for 10°C). This will ensure all batches are protected and that corrective action and preventive action (CAPA) is activated when the temperature exceeds the worst-case situation.

- **Risk Prioritization**: Once each processing step has been assigned an RPN, the task of prioritizing and performing mitigating actions begins. Management should initially accept the ranges (4) in Table II and, thereafter, any risks that remain at medium or high level for the individual process steps.
TABLE IV: Assigning a score to characterize the scenario for process material.

<table>
<thead>
<tr>
<th>Probability Factor</th>
<th>CFU Levels *</th>
<th>Water activity (See references 9, 10, 11)</th>
<th>Acidic pH (See references 10, 11, 12, 13)</th>
<th>Basic pH (See references 14, 15, 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Sterile **</td>
<td>&lt;2.0</td>
<td>≥10</td>
<td>≥60%</td>
</tr>
<tr>
<td>0.5</td>
<td>Very low content of CFU and in control</td>
<td>&lt;0.80</td>
<td>2.1 – 3.0</td>
<td>9.6 – 9.9</td>
</tr>
<tr>
<td>1</td>
<td>Low content of CFU and in control</td>
<td>0.81 – 0.89</td>
<td>3.1 – 4.5</td>
<td>9 – 9.5</td>
</tr>
<tr>
<td>1.5</td>
<td>Content of CFU in control</td>
<td>0.90 – 0.96</td>
<td>4.6 – 5.9</td>
<td>8 – 8.9</td>
</tr>
<tr>
<td>2</td>
<td>Unknown or uncontrolled conditions</td>
<td>0.97 – 1.00</td>
<td>6 – 7.9</td>
<td>0 – 2.9%</td>
</tr>
</tbody>
</table>

Note: If a material can fulfill more than several fields, then scientific judgements should be used for establishing the score.

*This level at the start of the process must be monitored adequately. Passing the solution through a bacteria-reducing filter (17) can be a way of achieving a low content of CFU at the beginning of the process.

**To achieve a zero score, the entire system needs to be sterile and process integrity must be ensured, so that there is no possibility of any bacterial growth.

***Alcohols in general are good preservatives, by either stopping microbiological growth or killing vegetative cells (14, 15).

Figure 4:
Flow diagram showing the principal steps.
Figure 5: Example of a partial API purification flow diagram.

USING THE QRM TOOL
The following are definitions of terms relevant to using the risk assessment tool.

Time
Time is defined as the period that the material (e.g., product, buffer, etc.) is either in contact with a specific piece of equipment or under processing. Time does not include what has occurred in the equipment before contact or processing has started, even though this period may allow micro-organisms to multiply and eventually contaminate the product.

Attributes of Process Material
Attributes of process material are a reflection of the product’s level of microbiological contamination at the start of the process step and its ability to support microbiological growth. The author’s experience showed that
assigning a score to characterise the scenario for process material can be challenging. Table IV was therefore developed at a later stage.

**Equipment Status and Attributes of Process Material**

If several handling steps occur in the same equipment, the equipment score will only be counted once (i.e., at the start). From the process example in Figure 7 the chromatography column score may, for example, have an equipment status score of 2 in step 2 that will then be 0 in step 3 (Figure 6).

**Risk Facilitators**

Risk facilitators should be used to ensure consistency in the use of the risk assessment tool. Requirements for being a facilitator must be established that include training of facilitator candidates by the following:

- Participating as an observer in assessments and facilitate assessments supervised by another facilitator
- Having the necessary negotiation skills
- A general education in QRM.

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**Figure 6:**
Equipment property scores if the same equipment is used in several steps.

<table>
<thead>
<tr>
<th>Process Step</th>
<th>2. Feed product to column</th>
<th>3. Binding product to column</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition of process material</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Added raw materials, buffers etc.</td>
<td>Score for (C) 1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Packaging Material</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Equipment Status (brief description)</strong></td>
<td>Column Z</td>
<td>Column Z</td>
</tr>
<tr>
<td><strong>Score for (E)</strong></td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

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**Figure 7:**
Example of chromatographic purification flow diagram.
Risk Assessment
Risk assessment requires a team of relevant experts (e.g., experts for the process being risk assessed and a microbiologist). Having operators who know the process involved in the risk assessment exercise is highly recommended, as there can be a difference between what is generally believed to happen and what actually happens.

Limitations of the Risk Assessment Tool
Limitations of the risk assessment tool should be taken into consideration. For example, even though a step has a hold time of more than 10 hours and an acceptable RPN, this does not mean it can be held indefinitely without being compromised due to microbiological growth. When in doubt, scientific judgement is required.

Detection of Bioburden
Detection of bioburden cannot be used as a factor to generate the RPN value because when bioburden and endotoxin are detected, it is too late.

THE CONTROL STRATEGY IN THE LIFECYCLE
Adhering to the risk assessment technique described here and using gathered knowledge continuously should prevent failures to occur during the products entire lifecycle. In the initial design and development phase for new processes, the RPN values should be used for design optimization to ensure that prevention of bioburden contamination is built into the design of the process.

During technology transfer, the initial gathered knowledge about the process and process material should be used for the design of the commercial manufacturing process. The tool can help from the initial design phase of the facility or equipment through the process validation to ensure that all sources of bioburden variation are accounted for before the commercial production begins. For commercial products, this tool will provide knowledge management during CAPA and change-management activities.

CONCLUSIONS
Using the risk management tool presented in this paper will give the following benefits with regards to bioburden control:

- Product quality is ensured
- Optimization of the production process sampling plan
- Significantly reduce time to perform the risk assessment
- QRM is performed in a consistent manor, despite varying processes and personal
- Essential knowledge is passed on easily during a products lifecycle, including technical transfer, change control, and CAPA activities
- Attending a risk assessment workshop and using the tool can be considered microbiological training within API production; that is fulfilling a GMP requirement (19).

The control strategy for the bioburden automatically shows up for every process step when we apply limits for the total risk score, thereby moving from risk assessment to risk management.

REFERENCES
1. FDA, 21 CFR §211 Current Good Manufacturing Practice For Finished Pharmaceuticals, Revised April 2008.
3. ICH, Q7 Good Manufacturing Practice Guide For Active Pharmaceutical Ingredients, November 10, 2000.
4. EMA, EU GMP Part II: Basic Requirements for Active Substances used as Starting Materials, February 3, 2010.
5. ICH, Q9 Quality Risk Management, November 9, 2005.

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Herman Herz, Ph.D., has 20 years experience within various pharmaceutical academic and business areas that include development of bio-production processes and consultancy. Dr. Herz has worked for Novo Nordisk as a microbiological senior expert, supporting production and product development.

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