Developing a comprehensive microbiological biosafety clearance strategy

A multifaceted approach

Verify that raw materials are free from contaminants and processes / environment & people are protected

Ensure Safety of Raw Materials and Processes

Develop capacity of manufacturing process with orthogonal steps that remove or inactivate contaminants

Implement Robust Clearance Technologies

Verify absence of contaminants in process intermediates

Optimize Sampling and Test Methodologies

Comprehensive Risk Mitigation Strategy
Risk assessment and mitigation strategies
Appropriate Single-use System Applications
Routes of contamination in the process
Filter categorization
Moderately critical filters and risk approach
Critical filters and risk approach
Filter qualification
**3D System Risk Assessment Concept**

**Used to calculate practical severity**

**Considers**
- System’s distance from the process stream
- Location along the process stream
- System’s complexity

Highest score is highest risk

\( \text{proximity} \times \text{location} \times \text{complexity} \)

This tool is mainly used to assign a risk level to an overall system **before** assessing failure frequency and detectability (SOD / RPN)

**Example of 3 Steps for Risk Assessment to Prevent Contamination**

1. **Identify**
2. **Mitigate**
3. **Detect**

*Excellent for complex systems as part of “big picture” analysis to prioritize risk management*

Risk Assessment: **Identify**

- **Mitigate**: Assess extent of risk, ability to detect, and frequency of occurrence
- **Identify**: Identify
- **Detect**: Detect

Each source is a potential entry point for microbial contamination such as:

- **Facility**
- **Equipment**
- **Process**
- **Materials**
- **Utilities**
- **Personnel**

- **Minute virus of mice (MVM)**: ~18-24 nm
- **Acholeplasma laidlawii**: < 0.2 µm
- **Leptospira species**: 0.4 µm x >>5 µm
- **Bacillus species**: 1 µm x 4 µm

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Case Studies of Microbial Contamination in Biologic Product Manufacturing
Suvarna, K.; Lolas, A.; Hughes, P.; Friedman, R. Biotechnology Manufacturing Team, Division of Manufacturing and Product Quality, Office of Compliance, Center for Drug Evaluation and Research, Food and Drug Administration
Map Out the Process Flow of Raw Materials

Each step may introduce microbes into the process

Handling
Transport of materials in the facility
Testing
Sampling
Transfer into different packaging
Storage conditions
Weighing
Sieving
Crushing
Sifting

Water transfer (cleaning, compounding)
Compounding
Mixing
Hold times
Dispensing
Sampling
Room Cleaning
Equipment Cleaning
Personnel Hygiene

How do I assess the risk of these parameters?

Risk Assessment: Mitigate

Identify
Mitigate
Detect

Eliminate source or reduce likelihood of occurrence
Prevent Human Contamination

Strategies for prevention, mitigation and detection

Prevention
- Remove people from the environment

Mitigation
When people have to be in the environment
- Wear cleanroom attire
- Work in cleanrooms
- Properly trained personnel

Detection
- Viable air sampling
- Surface monitoring
- Personnel monitoring

Prevent Raw Material Contamination

Raw Material Selection

Prevent
- Remove animal derived components
  - Caution! Serum-free does not mean mycoplasma free
  - Consider chemical free
- Select raw material quality grade
  - Pharmaceutical grade versus analytical grade
- Audit vendor

Mitigate
- Pre-treat components
  - Choose treatments effective for viral and bacterial reduction

Detect
- Screen raw material with rapid tests
  - Caution! Sample sizes versus kG to tons of material
### Considerations for bioreactor protection

#### Raw Material Pre-Treatment

<table>
<thead>
<tr>
<th>Technology</th>
<th>Robust Clearance</th>
<th>Media Compatibility</th>
<th>Point of Use</th>
<th>Scalability</th>
<th>Cost Effective</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTST (~100°C, ~10 sec)</td>
<td>Yes</td>
<td>Component dependent</td>
<td>Yes</td>
<td>Challenging for small to mid-scale</td>
<td>Yes at Large Scale</td>
</tr>
<tr>
<td>UV-C (254 nm)</td>
<td>Organism dependent</td>
<td>Component dependent</td>
<td>Yes</td>
<td>Challenging at large scale</td>
<td>Yes at Small Scale</td>
</tr>
<tr>
<td>γ Radiation</td>
<td>Organism dependent</td>
<td>Component dependent</td>
<td>No</td>
<td>Small batches</td>
<td>Yes</td>
</tr>
</tbody>
</table>

- **Downstream Virus Filters**: If specifically claimed, Consistent LRV
  - Yes but designed for downstream fluids
  - Yes
  - Yes
  - Not for batch processes*

- **Upstream Virus Barrier Filters**: Yes by size exclusion, Consistent LRV
  - Yes, specifically designed for upstream media
  - Yes
  - Yes
  - Yes

* Downstream viral clearance filters, are designed for very clean feed streams and would not be cost effective on upstream bioreactor media and feeds.

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### Key Points

#### Mitigate

- **Prevention**
  - Best option wherever possible

- **Containment**
  - Personnel Control
  - Single Use Technologies

- **Raw Material Selection**
  - Vendor qualification
  - Pre-treatment

- **Downstream Processing**
  - Microbiological Clearance
  - Filtration
  - Sterilization, sanitization, cleaning and storage
Risk Assessment: Detect

Classical Methods
Most developed in the 19th century
- Microscopy
- Growth-based methods

Benefits
- Easy to implement
- Easy to qualify
- Larger sample volumes possible

Limitations
- No universal medium or growth conditions
- Only detect those microbes capable of replicating in the chosen test medium under the specified conditions
- Can take days to weeks for a result

Rapid Methods
Developed over the past 30 years but slow adoption rate
- qPCR
- TMA
- Microcolony growth detection

Benefits
- Rapid results
- Higher sensitivity for equal volume compared to classical methods

Limitations
- More extensive validation
- Higher expertise required
- False positives doesn’t distinguish viable cells
- Small sample size
- Often destructive
  - Split samples needed for identification

“Contaminant-free” is only as good as the detection method used
**Limits of Detection**

**Sampling Volumes**

**Sampling**
- Vessel Liters to 10,000+ Liters
- Sample Volume
  - Less than 1 Liter

**Assay**
- Removed from sample volume
- Milliliter to microliter

**Sampling**
Assume a 1 L sample from a 10,000 L Bioreactor
Assay requires a 1 mL sample for testing

<table>
<thead>
<tr>
<th>CFU per Liter</th>
<th>10</th>
<th>1,000</th>
<th>10,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU per mL</td>
<td>0.01</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Probability an organism will NOT be detected in the sample</td>
<td>0.99</td>
<td>0.9</td>
<td>0.37</td>
</tr>
</tbody>
</table>

**Assay Sensitivity**

LOD PCR for *Leptospira*: 100 CFU (equivalent)
LOD PCR for Mycoplasma: 1-10 CFU (equivalent)
LOD by light microscopy @ 400 x: $10^5$ to $10^6$ cells
**Risk Assessment to Prevent Contamination**

- **Identify**
  - Assess extent of risk, ability to detect, and frequency of occurrence

- **Mitigate**
  - Eliminate source or reduce likelihood of occurrence

- **Detect**
  - Determine location frequency, and limit of detection

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**Some Risk Assessment Considerations for Filters**

- **Fluid classification**
  - Fluids labeled "sterile" have the highest risk

- **Dosage form**
  - Injectables without preservative have highest risk

- **Room classification**
  - Lower grade brings greater risk if there is a breach

- **Location of filter in the process**
  - The closer to the final product the greater risk

- **Detectability of poor filtration performance**
  - No in-line testing has the highest risk

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- **Contact time**
  - The longer the contact time the greater the risk

- **Process conditions**
  - The more aggressive the conditions, the greater the risk

- **Fluid pretreatment**
  - Less pretreatment has greater risk

- **Fluid posttreatment**
  - No downstream removal of low MW material has greater risk

- **Filter pretreatment**
  - The more aggressive the pretreatment (e.g. SIP), the greater the risk

- **Prior history**
  - If there have been previous filter related issues, the risk is greater
PDA Industry Aseptic Processing Survey 2001

**Personnel**
- Microbe Shedding  # 1
- Human Error  # 2
- Non-routine Activity  # 3
- Aseptic Assembly  # 4
- Mechanical Failure  # 5
- Routine AP Activity  # 7 (tie)
- Material Transfers  # 8 (tie)

**Environmental**
- Airborne Contaminants  # 6
- Surface Contaminants  # 7 (tie)
- Failure of HEPA Filter  # 8 (tie)

**Sterilisation**
- Improper Sanitization  # 7 (tie)
- Failure of 0.2 Filter  # 8 (tie)
- Improper Sterilization  # 9
Hierarchy of containment technology

- Automation/Robotics
- Isolators/Closed Transfer
- Directionalized Laminar Flow
- Laminar Flow
- Local Exhaust
- General Exhaust
- Open Operations

- Totally enclosed systems
- Secure connection technology
- RABS / Isolator technology

Single-use Products Offer Some Containment Opportunities

- Sterile Connectors and Disconnectors
- Assemblies, Support Containers and Mixers
- Systems
- Final Formulation and Filling Solutions

These products require components that need to be assembled
Example of Appropriate SUS Technology - Aseptic Sampling System

Increase sampling productivity, while reducing set-up, cleaning and flushing time whilst increasing biosafety.
Safe and easy aseptic sampling and disconnection directly from the container

Variety of sterile sampling devices and volumes including small volumes from 1 mL to 20 mL

Increases yield
Reduces sampling deviations
Reduces risk of non-sterility and reduced biosafety

Safe and easy sterile disconnection

Example of appropriate use of SUS technologies - multiple sterile connections
Previous SS System - Areas of Risk
Aseptic Build & SIP to Point-of-Filling

Connection under Laminar Air Flow

Opened connections

Advantages
- Ease of use
- Low cost

Challenges
- Connection needs to be performed in classified zone
- Risk of process design error
- Reduced risk of contamination
Sterile to sterile connectors

These connectors are designed to connect together sterile entities (container, tubing) in a non "classify A/B" environment.

Sterile to sterile connectors

What they do:

An operator independent and environment independent sterile connection between
Gamma sterilized assembly and Gamma sterilized assembly
Gamma sterilized assembly and Autoclaved assembly
Autoclaved assembly and Autoclaved assembly

Validated to maintain sterile flow path in any environment
Retention of Sterile-to-Sterile Connector
Aerosolized Testing of Connector in a Glove bag
(NB User environment would be grade C)

- Glove bag acts as isolator
- Suspension of *B. diminuta* is aerosolized into glove bag until total bacterial count is >$10^6$ cfu
- Connector is assembled and actuated under aerosolization
- Media transferred through connector under constant aerosolization of bacteria
- Media incubated and observed for turbidity over 7 days
- No turbidity or growth observed

SIP valve to Sterile Single Use lines

What they do:

Enables integration of Steamable hard piped process equipment with single-use sterile fluid paths

- Multi-steam, allowing for steam, disconnection, and re-steam
- Autoclaving
- Gamma compatible
- Validated to maintain flow path sterility
**Challenge: Joining Two Sterile Lines - Tube Welder**

A device where two sterile tubing lines are together heat welded. Tubing lines are inserted into holders. Then a heated blade cut the tubes allowing the lines to fuse together leaving a sterile fluid pathway.

**Challenge: Disconnecting Sterile Lines**

**Advantage**
- Ease to use

**Challenges**
- Single use
- Maintaining sterility after disconnections
- Speed of seal
- Dry or fairly dry
- Matching tubing or sleeve to sealer
- Moving equipment to tubing
SUS Example: Sterile Connect AND Reconnect

Multiple, sterile connections, disconnections and reconnections of single-use assemblies
Overview of Generic Biological Manufacturing Process

Microbiological Risk Focus

Regulatory and Compliance Microbiological Focus

Overview of Biopharmaceutical Process

Upstream

High microbiological risk
Little specific microbiological regulatory guidance

- Working Cell bank
- Seed Train
- Raw Materials
  - Water
  - Media Supplement
  - pH Adjusters
  - Antifoam
- Media Prep
- Mixing
- Pre-treatment Filtration
- Production Reactor > 500 L
- Clarification Filtration Centrifugation
- Harvest

- Sterility (Claim)
- Bioburden
- Virus
- Mycoplasma
- Endotoxin
Biopharmaceutical Process

**Downstream Purification (3)**

**Chromatography**
Anion exchange

**Virus**
Filtration

**Concentration**
Filtration

**Bulk Drug Substance**
In Single Use Containers

 Bulk Specification
< 10 CFU/ml

**Process Limit**
<X CFU (~ 20/ml)

**Sterility (Claim)**
**Bioburden**
**Virus**
**Mycoplasma**
**Endotoxin**

**Note – NOT claimed as sterile in this example**

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**Filterable Product Formulation / Filling Suite**

**High microbiological risk**
**Specific microbiological regulatory guidance**

**API**
WFI
Excipient

**Formulation**

**Prefilter Bioburden Sterilizing Filter**

**Sterile Hold Tank**

**Sterile**
Filtration

**Blanket / Transfer Gas Filter**

**Vent Filter**

**Utility Gas Filters**

**Gassing Filter**

**Blanket / Transfer Gas Filter**

**IT gas inlet**

**IT gas inlet filter**

**Vent Filter**

**Drying Filter**

**Washing Filters**

**Sterilizing Filter**

**Depyrogenation**

**Clean Room**

**Protection Filter**

**Filterable Product Formulation / Filling Suite**
Non-Filterable Product Formulation / Filling Suite

SUS Filling Assembly Example - SVP
Filters Can Be Divided into 3 Groups - Definitions

**Service**
The filter does not affect product quality
- Where process fluids come from facility-wide systems, are not tailored to a specific process and do not have contact with the drug substance or potential drug substance.
- Part of a No-Impact System - Where the equipment of system has no impact, direct or indirect, on product quality (ISPE Commissioning & Qualification Baseline Guide (2001))
  - Examples: distribution gas filter, water prefilter

**Moderately critical**
The filter indirectly affects product quality
- Where process fluids "will not be in direct contact with exposed sterile product or surfaces." (PDA TR40)
- Part of an Indirect Impact System - equipment or system expected to have incidental or secondary impact on product quality (ISPE Commissioning & Qualification Baseline Guide (2001))
  - Examples: vent filter in a grade D/C area, bioburden reduction filter

**Critical Applications**
The filter directly affects product quality
- Where process fluids "are in direct contact with sterile final product or critical surfaces of the associated equipment." (PDA TR40)
- Part of Direct Impact System - equipment or system that will have focused and immediate impact on product quality (ISPE Commissioning & Qualification Baseline Guide (2001))
  - Examples: vent filter on a sterile hold vessel, sterile liquid filter
Advantages of Classification - Multiple Process Lines or Bioreactor Trains

- Categorize each filter in a line based on risk, then duplicate across the whole production area.

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High Priority Filters in Product Formulation / Filling Suite
Purpose of Moderately Critical Filtration

Removal of undesirable microorganisms from process fluids
- Prevent contamination of the fermentation
- Cell culture media and air
- Formulation and process tanks
- Chromatography systems
- Buffers, washing fluids
- Process intermediates

Reduction of bioburden in purification process steps
- Low bioburden means low endotoxin
- Low / controlled / specified bioburden may be a compliance requirement
Buffers in a Generic Biologicals Process - moderately critical filtration

Closer to the formulation point, the higher the risk

Critical Filters and Risk Approach
Retention: What are the requirements for sterile medicinal products

"All Sterilization Processes Should be Validated."
WHO Annex 6: Good Manufacturing Practices for Sterile Pharmaceutical Products section 5.4 page 273

"Whatever type of filter or combination of filters is used, validation should include microbiological challenges to simulate “worst case” production conditions. The selections of the microorganisms to perform the challenge test (e.g. P. diminuta) has to be justified. The nature of the product may affect the filter and so the validation should be performed in the presence of the product....."
PIC/S Guide for Inspectorates: Recommendation on the Validation of Aseptic Processes

A summary should be provided containing information and data concerning the validation of the retention of microbes and compatibility of the filter used for the specific product.
US FDA Guidance on Sterilization Validation

What Critical Filters need to be Qualified for a Sterile Medicinal Product

Sterilizing liquid filter
Bioburden reduction filter
Sterilizing gas filtration

Note: 0.22 or 0.2 or 0.1um rated filters may not be sterilizing grade.
Sterilizing grade filters are specifically labelled, qualified and documented. This is what to look for when choosing a critical filter.
8 Elements of Sterile (Critical) Filter Qualification
Represent "worst case" process conditions, process fluid, filter performance and microbiological challenge

- Prove the filter’s bacterial retention capabilities with a non-destructive test.
- Prove the filter removes bacteria from the stream compliant with ASTM 838-05 and regulations.
- Prove the stream does not adversely impact the filter duty or process stream.
- Identify, quantify, and assess impact of compounds that migrate from filter to process stream.
- Prove the sterilization method is effective and does not compromise the filter.
- Prove the stream does not unacceptably remove stream components.
- Prove the filter meets all performance & duty requirements within product & process conditions.
Requirements for Filter Documentation

✓ Suitability for duty
✓ Process definitions
✓ Bacterial / particulate retention
✓ Integrity testing
✓ Sterilisation process validation
✓ Adsorption
✓ Leachables / Extractables
✓ Risk analysis approach to processing and product impact
✓ Quality by design

Thank You for your Attention!
May we be of Further Assistance?

michael.payne@merckgroup.com
For biological materials that cannot be sterilized (e.g. by filtration), processing must be conducted aseptically to minimise the introduction of contaminants.

5. As part of the control strategy, the degree of environmental control of particulate and microbial contamination of the production premises should be adapted to the active substance, intermediate or finished product and the production step, bearing in mind the potential level of contamination of the starting materials and the risks to the product.

6. Manufacturing and storage facilities, processes and environmental classifications should be designed to prevent the extraneous contamination of products. Prevention of contamination is more appropriate than detection and removal.

8 c) Live organisms and spores are prevented from entering non-related areas or equipment by addressing all potential routes of cross-contamination and utilizing single use components and engineering measures such as closed systems.

8 e) Environmental monitoring specific for the micro-organism being manufactured, where the micro-organisms are capable of persistence in the manufacturing environment and where methods are available, is conducted in adjacent areas during manufacture and after completion of cleaning and decontamination.

8 f) Products, equipment, ancillary equipment (e.g. for calibration and validation) and disposable items are only moved within and removed from such areas in a manner that prevents contamination of other areas, other products and different product.
Annex 2 Manufacture of Biological active substances and Medicinal Products for Human Use

13. Equipment used during handling of live organisms and cells, including those for sampling, should be designed to prevent any contamination during processing.

16. Air vent filters should be hydrophobic and validated for their scheduled life span with integrity testing at appropriate intervals based on appropriate QRM principles.

33. Given that the risks from the introduction of contamination and the consequences to the finished product is the same irrespective of the stage of manufacture, establishment of a control strategy to protect the product and the preparation of solutions, buffers and other additions should be based on the principles and guidance contained in the appropriate sections of Annex 1.

34. Where sterilization of starting and raw materials is required, it should be carried out where possible by heat. Where necessary, other appropriate methods may also be used for inactivation of biological materials (e.g. irradiation and filtration).

51. The growth promoting properties of culture media should be demonstrated to be suitable for its intended use. If possible, media should be sterilized in situ. In-line sterilizing filters for routine addition of gases, media, acids or alkalis, anti-foaming agents etc. to fermenters should be used where possible.

52. Addition of materials or cultures to fermenters and other vessels and sampling should be carried out under carefully controlled conditions to prevent contamination. Care should be taken to ensure that vessels are correctly connected when addition or sampling takes place.