Designing Critical Cleaning Processes to Mitigate Microbial Risk in Multiproduct Facilities

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Agenda

• Designing critical cleaning parameters
• Understanding microbes and resistance
• Cleaning conditions to control microbes
• Case History - New Product Introduction: Cleaning and Microbiological Considerations

Disinfectant regulations

• All germicidal cleaners in the United States fall under FIFRA as amended (1988) and administered by EPA

• Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)

• FDA regulation as medical device per Food Quality Protection Act of 1996 - if used to reprocess other medical devices or if used as a sterilant for medical devices

• EPA governs the product safety, use, efficacy, disposal, and label
“Microorganism”

- "Yeasts, molds, bacteria, viruses or other similar microscopic organisms"
- Includes species that:
  - May have public health or sanitary significance
  - May cause product to decompose
  - May be indication that product contaminated with gross soil
  - May cause product to be adulterated
- CGMP requirements emphasize cleaning and control of microbes

Designing critical cleaning parameters

Evaluate current cleaning practices for effective removal of
- Product residues
- Process residues
  - Cell debris, impurities, microbes, viruses, DNA, lipids, proteins, peptides, buffer, salts ………
- Cleaning agent(s) residues

Cleaning Critical Parameters:
- Time (wash, rinse, etc.)
- Action
- Cleaning agent
- Cleaner concentration
- Temperature (wash, rinse, etc.)
- Water quality
- Environmental factors (e.g., humidity)
- Rinsing

Designing critical cleaning parameters

- Cleaning coverage (riboflavin)
- Valves, gaskets and tubing
  - Inspect for indication of wear
- Corners and crevices
- Materials of construction and roughness


Rouge and water scale
- Dead legs
- Connections
- Flow rates in pipes
- Slope/Drainability


Residue and soil type

Summary of Factors Affecting Cleaning

Potential sources of microbes

- Raw materials
  - actives and non-actives
- Packaging components
- Personnel
- Environment
  - air, room
- Utilities
  - water
- Equipment

Potential sources of microbes

Supplier/raw material selection, pre-setting microbial limits or sterilizing them

Establishing cleanroom disinfection, gowning procedures, training and environmental monitoring

Qualifying and maintaining water generation system, compressed air, \( \text{N}_2 \), etc

Establishing robust cleaning procedures, addressing design issues, and setting sterilization or sanitization parameters

Process control of microbial contamination

Simplified manufacturing process

Bulk MFG
- Crude separation
- Capture and Polishing
- Chromatography/UF/DF
- Viral clearance
- inactivation/filtration
- Purified bulk

Process design identifies risks to product and builds in controls

- Selection/sourcing
- Gamma irradiation
- Ultraviolet
- Chaotropic salts
- \( \text{pH} \) inactivation
- Solvent-detergent treatment
- Heat treatment
- Chromatography
- Filtration

Multiproduct facilities can present new risks to defined processes

Understanding resistance hierarchy

Prions (BSE/TSE)
Bacterial spores
Viruses (non-enveloped)
Mycobacteria
Bacteria (Gram -)
Endotoxins
Biofilms
Bacteria (Gram +)

Understanding resistance hierarchy

Growth conditions, treatment, and sterilization: types, actions, and resistance. 2007, ASM Press
Estimated survival times of microbes

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Days</th>
<th>Weeks</th>
<th>Months</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycoplasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enveloped virus (avian influenza)</td>
<td></td>
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<tr>
<td>Gram positive bacteria (Staphylococcus)</td>
<td></td>
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<tr>
<td>Gram negative (Brucella)</td>
<td></td>
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<tr>
<td>Mycobacteria (Mycobacterium bovis)</td>
<td></td>
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<tr>
<td>Fungal spores (Dermatophytes)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Non-enveloped virus (feline parvovirus)</td>
<td></td>
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<tr>
<td>Prions (BSE)</td>
<td></td>
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<td></td>
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<tr>
<td>Endospores (Bacillus anthracis)</td>
<td></td>
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</tr>
</tbody>
</table>

Antimicrobial spectrum of chemical disinfectants

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Gram +</th>
<th>Gram -</th>
<th>Myco-bacteria</th>
<th>Endo-spores</th>
<th>Fungal spores</th>
<th>Viruses - enveloped</th>
<th>Viruses - non-enveloped</th>
<th>Prions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acids (inorganic)</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Alcohol</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aldehyde</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Alkali</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ageredies</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Halogenated (chlorine)</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Peroxymid acid</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Phenol</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>QACs</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

++ = Highly effective, + = effective, _ = limited activity, _ = no activity

Bacteria control

A formulated alkaline detergent is registered with the Environmental Protection Agency (EPA) as a broad spectrum disinfectant.

Effective against Staphylococcus aureus, Salmonella choleraesuis and Psuedomonas aeruginosa when used at 1% w/v concentration in water with 5% blood serum and 250 ppm of hard water at 60°C for at least 5 minute contact time.

NOTE: The critical parameters and factors that can affect microbial efficacy with chemical disinfectants are Concentration, Time, Temperature, Surface, Biomass, Soil Levels and Water Hardness.
**Endotoxin reduction**

General approach: sodium or potassium hydroxide alone or in a formulated cleaner, dry heat or positively charged 0.22 micron filtration.

5% w/w formulated alkaline detergent at 60°C for 5 minutes provided a > 3 log better reduction than water alone from glass surfaces.

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**Mycoplasma control**

Mycoplasma was dried on stainless steel surfaces (<0.5 log10 reduction of efficacy upon drying) and exposed for 5 minutes at 30°C with a formulated alkaline detergent.

<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>0.2%</th>
<th>0.4%</th>
<th>0.8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acholeplasma laidlawi</td>
<td>0.8 ± 0.2</td>
<td>3.5 ± 0.9</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td>Mycoplasma gallisepticum</td>
<td>1.0 ± 0.1</td>
<td>3.8 ± 1.2</td>
<td>&gt;4.5</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>1.4 ± 0.1</td>
<td>3.6 ± 0.7</td>
<td>&gt;4.5</td>
</tr>
</tbody>
</table>

The article also reported a >4.5 log reduction with 70% Ethanol, <0.5 log reduction with QAC at 0.05% active. VHP exposure at various concentrations and time was also reported.

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**Viral inactivation**

Inactivation of Adenovirus Type 5 constructs with 10 minute exposure at room temperature.

<table>
<thead>
<tr>
<th>Sample</th>
<th>0.09M NaOH</th>
<th>0.9% Formulated alkaline detergent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified construct 1</td>
<td>6.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Purified construct 2</td>
<td>&gt;7.9</td>
<td>&gt;8.8</td>
</tr>
<tr>
<td>Purified construct 3</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Unpurified construct 1</td>
<td>6.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Unpurified construct 2</td>
<td>6.0</td>
<td>6.5</td>
</tr>
<tr>
<td>Unpurified construct 3</td>
<td>7.2</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Risat Jannat et al., (Merck Research Laboratories), Biotechnol. Prog. 2005, 21, 446-450
**Viral inactivation**

Virucidal activity against viruses dried on stainless steel surfaces with a 10 minute exposure time at ambient temperature unless noted.

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Formulated alkaline detergent, 0.9%</th>
<th>Ethanol, 70%</th>
<th>QAC, 0.05%</th>
<th>Hypochlorite, 7.5%</th>
<th>Sodium hypochlorite, 2500 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine Parvovirus</td>
<td>4.9±0.3</td>
<td>0.6±0.3</td>
<td>0.5±0.3</td>
<td>0.5±0.3</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Minute Virus of Mice</td>
<td>&gt;4.4</td>
<td>0.7±0.1</td>
<td>0.5±0.2</td>
<td>1.5±0.4</td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>Poliovirus (sabin)</td>
<td>&gt;4.6</td>
<td>1.8±0.2</td>
<td>0.8±0.1</td>
<td>3.5±0.1</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Adenovirus type 5</td>
<td>&gt;4.1</td>
<td>1.2±0.1</td>
<td>1.2±0.1</td>
<td>2.2±0.2</td>
<td>&gt;4.1 (1 min)</td>
</tr>
<tr>
<td>Vaccine virus</td>
<td>&gt;5.0</td>
<td>&gt;5.0</td>
<td>3.2±0.2</td>
<td>4.9±0.1</td>
<td>&gt;4.6 (1 min)</td>
</tr>
</tbody>
</table>

Note: Other active components, formulations, dry heat and moist heat were evaluated in the study.

Eterpi, M., et al., Journal of Hospital Infection (2009) 73, 64-70

**Viral inactivation**

A formulated alkaline detergent is registered with the Environmental Protection Agency (EPA) as a virucidal agent.

Effective against Poliovirus (type 1) at 1% v/v formulated alkaline detergent, 5% blood serum, 250 ppm hard water at 60°C for at least 10 minute contact time.

**Prion inactivation**

- Novel methods for disinfection of prion-contaminated medical devices
  - Lancet 2004; 364: 521-26
  - Guillaume Fichet et al.
  - 1.6% Formulated alkaline detergent, 43°C for at least 15 min
- Degradation and destabilization of abnormal prion protein using alkaline detergents and proteases
  - International Journal of Molecular Medicine 25: 267-270, 2010
  - Yoshihiko Hirata et al.
  - 1.6% Formulated alkaline detergent, 43°C for at least 15 min
Summary

- Effective cleaning
  - Removal of process, product, and cleaning agent residues
- Equipment designed for cleanability
  - Coverage, drainability, minimize dead legs, valve selection, etc...
- Review cleaning parameters against microbial control parameters (such as time, temperature, concentration, cleaning agent and cleaning method) and assess risk of microbial cross contamination from one batch or product to another

CASE HISTORY
New Product Introduction: Cleaning and Microbiological Considerations
Mary Ellen Clark
Validation Scientist
MedImmune

New Product Introduction

- Several key elements are critical when introducing a product into a facility to mitigate risk:
  - Product Impact Risk Assessment
    - Toxicological Assessment
    - Regulatory
    - Agency reporting requirements (commercial facilities)
    - Employee Safety
    - Facility Capability & Compatibility
    - Potential Biological Contamination (e.g., viral load for Mabs)
  - Product & Process Component Cleanability
    - In-house Evaluation
      - Study Design
      - Selection of Materials to include in study (availability)
      - Justification of selected materials
      - Testing Requirements
      - Report
    - Out-sourced Evaluation
      - Can be performed by a qualified testing lab
      - Can be used to supplement in-house data
New Product Introduction, Continued

• Cleanability continued:
  • Recovery
    – Use a standardized approach
    – Study required to ensure that process solutions, (actives as well as potential process intermediates), can be recovered and detected
  • Use of the validated assay(s)
• Solubility
  – New Buffer Components associated with the incoming product should be identified and included in the risk assessment
  – Comparison to previous solutions utilized in the facility
• Product Inactivation
  – Use a standardized approach
  – Study required to ensure that process solutions (proteins) are in fact denatured by the cleaning chemicals and not reversible.
  – Selection of the detection assay

Best Practices when Designing a Cleaning Program

• When designing a CIP program that enables a facility to operate in multi-product campaign mode the following should be considered:
  – Process Stream Types
  – Chemical Compatibility
  – Time and Temperature Requirements
  – Hold Time Requirements (clean and dirty)
  – Robust cycle development
  – Equipment Design
  – Points of potential contamination sources

• Having a strategy documented prior to the CIP development phase of a project will provide the foundation to which all building blocks are applied. Microbial control in a large scale manufacturing facility is multi-faceted and when implemented correctly changeover can be executed in a timely and consistent manner.

Cleaning Strategies in a Multi-Product Environment

The following diagram represents an example of a skid which is used for Multi-Product Operations.

The following case study outlines an event which occurred during Multi-Product cleaning operations.
Case Study

- A bioburden and endotoxin excursion was detected on rinse samples from a processing skid. These samples were obtained during routine revalidation activities.
  - A deviation was initiated.
  - An investigation was performed to determine the root cause of the failure.
  - Corrective actions were initiated.
- The investigation team focused on ensuring that all aspects associated with the cleaning event were investigated.

Potential Sources included:
- Personnel
- Equipment
- Automation Issues
- Process Solutions
- Cleaning Methods
- Cleaning Solutions
- QC Monitoring Staff
- Artifact of Test Method

Case Study, Continued

- A cross functional team was implemented to perform an investigation and compile data.
  - The Team evaluated the following areas:
    • Results of all rinse and swab samples
    • CIP Skid functionality
    • Equipment Design, Operation, & Maintenance
    • QC monitoring techniques
    • CIP recipe design
    • Dirty Hold duration
    • Process Soilant / Operations
    • Change Control History

Case Study, Conclusion

- What did the team find?
  - A water-for-injection drop used as a source of water to dilute the in-process material was found to be contaminated with the same bacteria as the revalidation sample (bacillus). Corrective actions were initiated including updating the PM procedure.
  - Due to the overloading of the system with bioburden and a long dirty hold, the CIP was not able to adequately able to chemically sanitize the system. (The original validation study was not executed with contaminated solvents).
  - An evaluation of the process performed using the skid indicated that equipment handling improvements can be implemented to further mitigate risk of future contamination.
    • The filters associated with the system will be sanitized via autoclave prior to use (To eliminate a potential microbiological source).
    • A WFI rinse will be performed following completion of use.
    • The equipment and its contents will be SIPd (sanitized) prior to disassembly.
    • An air blow of the system will be performed to reduce residual liquids (drying and cooling).
    • The room will be cleaned immediately following disassembly to prevent contamination to the room.
Conclusion

• When developing a validation study operational events can be anticipated and built into the study protocol.

• Microbiological issues can arise within a validated process.

• A revalidation or verification program should be incorporated into your routine and changeover programs.