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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

Residue and Soil type ⟺ Composition, Amount, Condition, Dirty hold time

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

Designing critical cleaning parameters

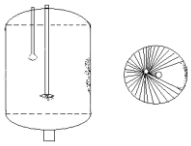
Cleaning Critical Parameters:

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

Verghese, G. (1998). Selection of Cleaning Agents and Parameters for cGMP Processes, *Proceedings of the INTERPHEX Conf.*, Philadelphia, Reed Exhibition Co, Norwalk, CT, pp. 89-99.

Designing critical cleaning parameters

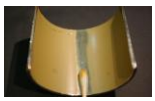
- Cleaning coverage (riboflavin)
- Valves, gaskets and tubing
  - Inspect for indication of wear
- Corners and crevices
- Materials of construction and roughness



Rivera. E. "Basic Equipment Design Concepts to Enable Cleaning in Place". Pharmaceutical Technology. http://pharmtech.findpharma.com/pharmtech/article/articleDetail.jsp?id=726190

Designing critical cleaning parameters

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



Lopolito, P. " Addressing Rouge in Biopharmaceutical Manufacturing". Pharmaceutical Technology. http://pharmtech.findpharma.com/pharmtech/article/articleDetail.jsp?id=686036

Designing critical cleaning parameters

Summary of Factors Affecting Cleaning

G. Verghese and P. Lopolito, "Cleaning Engineering and Equipment Design," in *Cleaning and Cleaning Validation*, P. Pluta, Ed. (DHI Publishing, River Grove, IL, vol. I, 2009), pp. 123–150

Potential sources of microbes

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

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

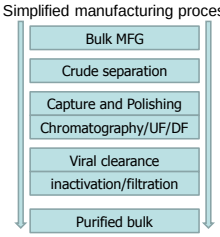
Establishing cleanroom disinfection, gowning procedures, training and environmental monitoring

Qualifying and maintaining water generation system, compressed air, N_2, etc

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

Process control of microbial contamination

Simplified manufacturing process



Process design identifies risks to product and builds in controls

- Selection/sourcing
- Gamma irradiation
- Ultraviolet
- Chaotropic salts
- pH inactivation
- Solvent-detergent treatment
- Heat treatment
- Chromatography
- Filtration

Multiproduct facilities can present new risks to defined processes

Understanding resistance hierarchy

	Microorganism	Examples
More Resistant	Prions	Scrapie, Creutzfeldt-Jacob disease, Chronic wasting disease
	Bacterial Spores	*Bacillus, Geobacillus, Clostridium*
	Protozoal Oocysts	*Cryptosporidium*
	Helminth Eggs	*Ascaris, Enterobius*
	Mycobacteria	*Mycobacterium tuberculosis, M. farnae, M. chelonae*
	Small, Non-Enveloped Viruses	Poliovirus, Parvoviruses, Papilloma viruses
	Protozoal Cysts	*Giardia, Acanthamoeba*
	Fungal Spores	*Aspergillus, Penicillium*
	Gram negative bacteria	*Pseudomonas, Providencia, Escherichia*
	Vegetative Fungi and Algae	*Aspergillus, Trichophyton, Candida, Chlamydomonas*
	Vegetative Helminths and Protozoa	*Ascaris, Cryptosporidium, Giardia*
	Large, non-enveloped viruses	Adenoviruses, Rotaviruses
	Gram positive bacteria	*Staphylococcus, Streptococcus, Enterococcus*
Less Resistant	Enveloped viruses	HIV, Hepatitis B virus, Herpes Simplex virus

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

Gerald McDonnell, "Antisepsis, Disinfection, and Sterilization: Types, Action, and Resistance" 2007, ASM Press

Estimated survival times of microbes

Microbe	Days	Weeks	Months	Years
Mycoplasma	→			
Enveloped virus (avian influenzavirus)	→			
Gram positive bacteria (*Staphylococcus*)	→	→		
Gram negative (*Brucella*)	→	→		
Mycobacteria (*Mycobacterium bovis*)	→	→		
Fungal spores (*Dermatophytes*)	→	→	→	
Non-enveloped virus (*feline parvovirus*)	→	→	→	
Prions (BSE)	→	→	→	→
Endospores (Bacillus anthracis)	→	→	→	→

Quinn, J.P. Chapter 54 Disinfection and Disease Prevention in Veterinary Medicine, page 1077; Disinfection, Sterilization and Preservation; 5th Edition by Seymour S. Block

Antimicrobial spectrum of chemical disinfectants

	Gram +	Gram -	Myco-bacteria	Endo-spores	Fungal spores	Viruses - enveloped	Viruses – non enveloped	Prions
Acids (mineral)	++	+	_	±	±	+	±	_
Alcohols	++	++	++	_	+	+	_	_
Aldehydes	++	+	+	++	++	++	++	_
Alkalis	++	++	+	+	+	+	+	+
Biguanides	++	+	_	_	+	+	_	_
Halogens (chlorine)	++	++	+	+	+	++	++	±
Hydrogen peroxide	++	++	±	+	+	++	±	_
Peracetic acid	++	++	++	++	++	++	+	_
Phenols	++	++	+	_	+	+	_	_
QACs	++	+	_	_	+	+	_	_

++, Highly effective; +, effective; ±, limited activity, _, no activity

Quinn, J.P. Chapter 54 Disinfection and Disease Prevention in Veterinary Medicine, page 1077; Disinfection, Sterilization and Preservation; 5th Edition by Seymour S. Block

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% v/v concentration in water with 5% blood serum and 250 ppm of hard water at 60^{0}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, Bioburden, 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

Mycoplasma control

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

Mycoplasma	0.2 %	0.4 %	0.8%
Acholeplasma laidlawi	0.8 ± 0.2	3.5 ±0.9	>4.5
Mycoplasma gallisepticum	1.0 ± 0.1	3.8 ±1.2	>4.5
Mycoplasma pneumoniae	1.4 ± 0.1	3.6 ±0.7	>4.5

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.

Eterpi, M. et al. "Decontamination Efficacy Against Mycoplasma" Letters in Applied Microbiology, 52, 150-155 (2010) ISSN 0266-8254

Viral inactivation

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

Sample	0.09M NaOH	0.9% Formulated alkaline detergent
Purified construct 1	6.7	7.3
Purified construct 2	>7.9	>8.0
Purified construct 3	7.8	7.8
Unpurified construct 1	6.5	6.2
Unpurified construct 2	6.0	6.5
Unpurified construct 3	7.2	6.6

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

	Formulated alkaline detergent, 0.9%	Ethanol, 70%	QAC, 0.05%	Hydrogen peroxide, 7.5%	Sodium hypochlorite, 2500 ppm
Porcine Parvovirus	4.9±0.3	0.6±0.3	0.4±0.3	0.5±0.1	1.0±0.1
Minute Virus of Mice	>4.4	0.7±0.1	0.5±0.2	1.5±0.4	4.4±0.1
Poliovirus (sabin)	>4.6	1.8±0.2	0.8±0.1	3.9±0.1	4.5±0.2
Adenovirus type 5	>4.1	>4.1	1.2±0.1	2.3±0.2	>4.1 (1min)
Vaccina virus	>5.0	>5.0	3.2±0.2	4.9±0.1	>4.6 (1min)

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

Eterpi, M., et al., Applied Biosafety (2010) vol.15, no 4, 165-171
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 60ºC 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, 43ºC 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 , 43ºC 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 at 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

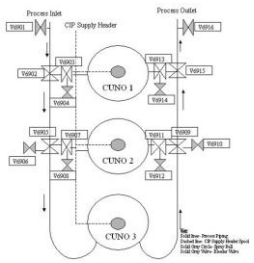
Best Practices when Designing a Cleaning Program

- 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 soilants).
  - 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.