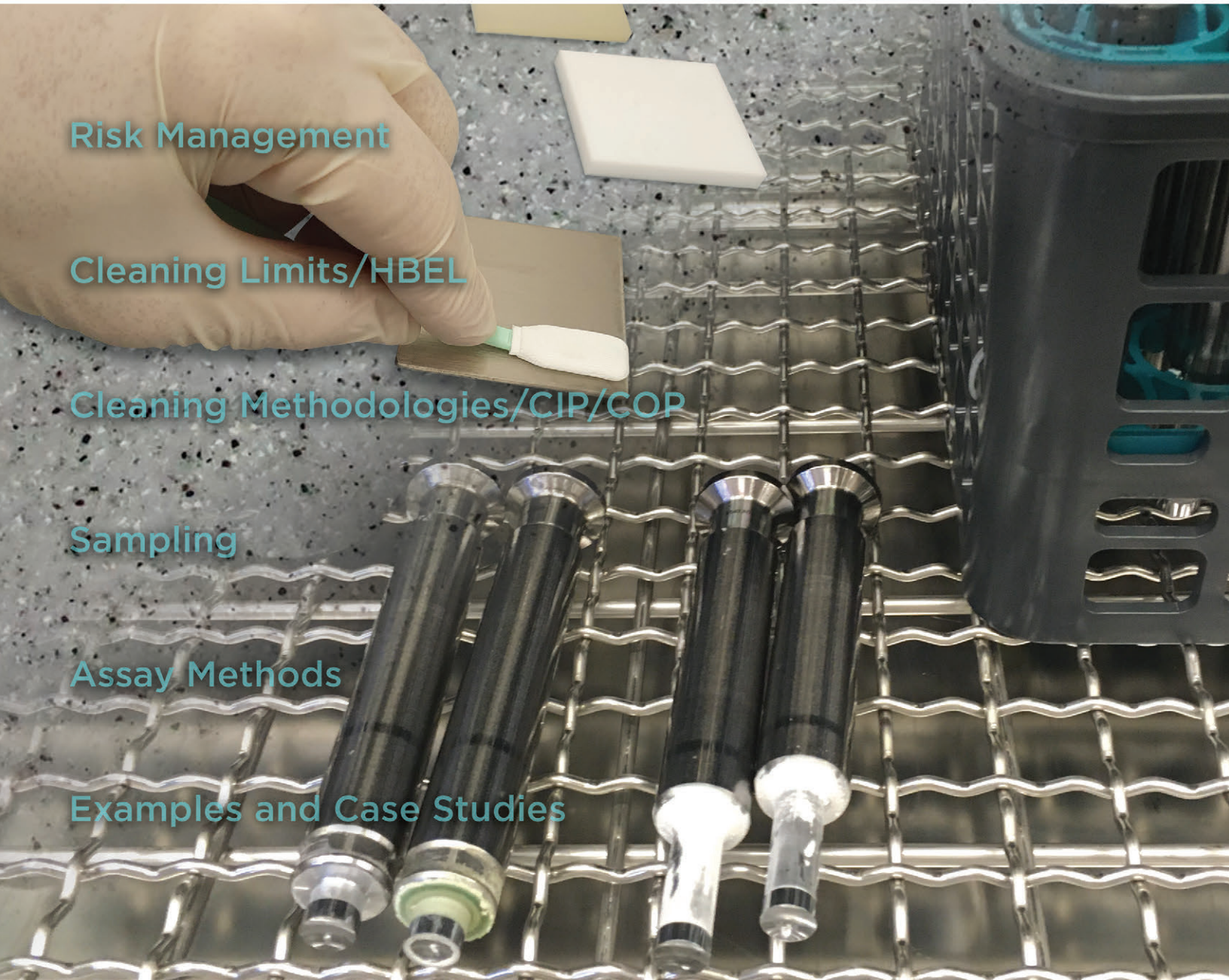
ISPE®

GUIDE:

Cleaning Validation Lifecycle – Applications, Methods, and Controls



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Cleaning Validation Lifecycle – Applications, Methods, and Controls

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Preface

Regulatory agencies expect the development and validation of a compliant cleaning program. This critical activity ensures that the risks of contamination, product carryover, and cross-contamination are controlled, minimized, and monitored to safeguard patient safety and product quality.

This *ISPE Guide: Cleaning Validation Lifecycle – Applications, Methods, and Controls* describes the application of the process lifecycle model to cleaning. This will aid organizations in developing and adopting scientifically sound approaches, resulting in a robust cleaning validation program.

An integral part of an effective cleaning program is using risk-based approaches in the design and management of the validation process; accordingly, this ISPE Guide is aligned with the principles described in the *ISPE Baseline® Guide: Volume 7 – Risk-Based Manufacture of Pharmaceutical Products [Risk-MaPP] (Second Edition)*.

This *ISPE Guide: Cleaning Validation Lifecycle – Applications, Methods, and Controls* promotes the use of health-based exposure limits (HBEL) and offers guidance and examples for developing and/or transitioning to the determination of cleaning specifications using HBEL.

Created by a team of industry experts with global experience, this ISPE Guide is intended as a reference for the cleaning lifecycle model as well as a practical guide for applying the theory and concepts to help create compliant and effective cleaning programs.

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The Guide was produced by a Task Team led by Jose Caraballo (Bayer U.S., USA) and Joseph Payne (Tergus Pharma (formerly with Alcami Corp.), USA).

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1 Introduction

Cleaning validation is a requirement of the biotechnology, biological, pharmaceutical, diagnostics, medical device, nutraceutical, and in some cases cosmetic industries. Regulatory agencies expect the development and validation of a compliant cleaning program. This critical activity ensures that the risks of contamination, product carryover, and cross-contamination are controlled, minimized, and monitored to safeguard patient safety and product quality.

An effective cleaning program shall be in place to provide documented evidence that cleaning processes will reproducibly remove previous product or other residues on product contact equipment surfaces below scientifically set acceptable levels [1]. Cleaning soiled surfaces allows the use of that equipment with other products or materials without undue risk of cross-contamination of those products or materials.

This *ISPE Guide: Cleaning Validation Lifecycle – Applications, Methods, and Controls* aims to provide a comprehensive explanation and hands-on guidance for cleaning validation lifecycle. It describes the fundamental elements applicable to the pharmaceutical, biotechnology, and other life science-related industries. This ISPE Guide has been created as a consensus document by a team of industry experts after careful consideration of regulatory requirements, industry practices, and feedback received during peer review. Alternate approaches to the ones described here to meet regulatory requirements may be acceptable if scientifically justified and properly documented. A country, company, or facility may have additional or unique requirements that could necessitate adaptation of the principles presented in this ISPE Guide on a case-by-case basis.

1.1 Background

The main purpose of cleaning validation is to prove the effectiveness and reproducibility of the cleaning process for a given piece of production equipment to prevent cross-contamination and adulteration of a Drug Product (DP), substance, or biological product from other active ingredients, chemicals, and other unintended compounds or microbiological contamination. It also establishes criteria to reduce patient risk by producing a safe and effective product.

Cleaning validation is labor intensive, requiring resources from multiple areas. Functions involved in cleaning activities include:

- Research and Development (e.g., establishment of manufacturing processes and corresponding cleaning agents)
- Process Development (e.g., establishment of cycle development studies, calculations)
- Toxicology (to determine Health-Based Exposure Limits (HBELs) and provide Acceptable Daily Exposure (ADE)/ Permitted Daily Exposure (PDE) information as needed)
- Virology (when cleaning biologicals and equipment used for advanced therapies)
- Engineering (for equipment design and readiness)
- Production (e.g., operating equipment, supporting validation execution, troubleshooting failures)
- Validation (e.g., writing validation protocols, reports, and master plans, and/or executing validation protocols)
- Quality Control (e.g., sampling, laboratory recovery studies, methods development, and validation studies)
- Quality Assurance (e.g., ensure compliance with GMP regulations)

Over the years regulated GxP industries have acknowledged that cleaning validation is an integral part of manufacturing operations and should be treated as a critical process. Applying a process lifecycle model to cleaning processes helps ensure the application of scientifically sound approaches, resulting in a robust cleaning validation program.

1.2 Purpose and Objectives

1.2.1 Benefit

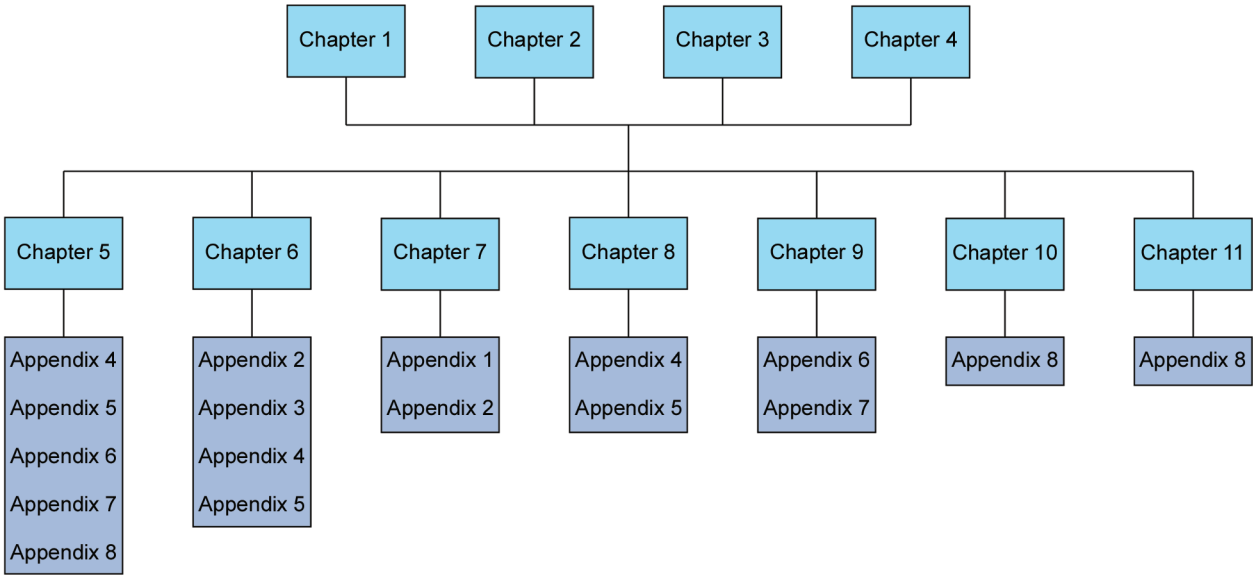
This Guide provides a hands-on approach to support the pharmaceutical, biotechnology, and other life science industries in the development and establishment of compliant cleaning programs that will meet or exceed regulatory expectations. This Guide is intended as a reference for the cleaning lifecycle model and a practical guide for applying the theory and concepts to product contact surfaces to help create compliant and practical cleaning programs.

1.2.2 Scope

Application of phased appropriate GMPs for commercial and clinical manufacturing cleaning practices (validation or verification) is addressed in this Guide.

As illustrated in Figure 1.1, this Guide is designed in three parts. Chapters 1 to 4 provide an overview, containing industry trends, regulatory references, cleaning risk management, and the cleaning validation lifecycle. The reader is then led through the validation lifecycle stages. Chapters 5 to 11 present in-depth information on planning the process, preparing and conducting validation, and implementing and maintaining the validated processes. These chapters provide guidance for creating new programs as well as bringing legacy programs in line with current regulatory expectations. This information culminates into several examples and case studies (Appendices 1 to 8) to help increase the reader's understanding of the application of these principles in the cleaning process.

Figure 1.1: Guide Structure

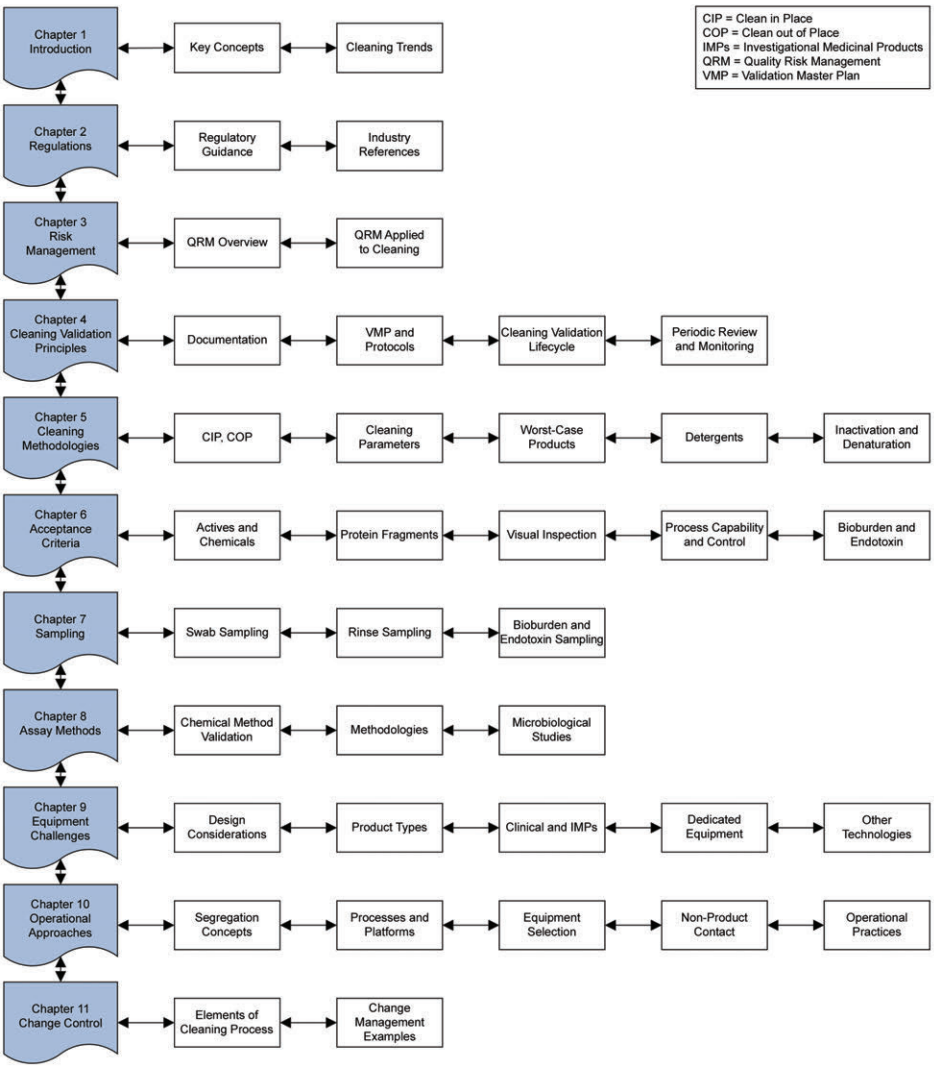


The Guide discusses the following topics (see also Figure 1.2):

- Current regulations and relevant standards
- Application of risk management in cleaning validation
- Description of the cleaning validation lifecycle model

- Cleaning methodologies
- Creation of cleaning validation acceptance criteria
- Determination of visual inspection limits
- Cleaning validation strategies
- Documentation for cleaning validation programs
- Calculation and justification of residue limits
- Validation of testing and sampling methods
- Periodic review and revalidation
- Equipment issues and challenges
- Examples and case studies

Figure 1.2: Chapter Organization of this Guide



This Guide pertains to pharmaceutical, biotechnology, and other life science industries with the exception of medical devices. Cleaning validation requirements for medical devices are contained in ASTM F3127 – 16, Standard Guide for Validating Cleaning Processes Used During the Manufacture of Medical Devices [2].

Also excluded from this Guide are clean rooms cleaning and controls, and facility cleaning and disinfection.

A short discussion on indirect and non-product contact surfaces is in Section 10.4; however, they are not within the scope of this Guide. See the *ISPE Baseline® Guide: Volume 7 – Risk-Based Manufacture of Pharmaceutical Products [Risk-MaPP] (Second Edition)* [3] for developing control strategies to prevent cross-contamination of any surface.

1.3 Key Concepts

Cleaning validation, according to Eudralex Volume 4, Annex 15: Qualification and Validation [4] is:

> *"Documented evidence that an approved cleaning procedure will reproducibly remove the previous product or cleaning agents used in the equipment below the scientifically set maximum allowable carryover level."*

Risk-based approaches are an integral part of cleaning validation. The evaluation of hazards, the analysis of risks, and the mitigation of those risks with control measures using risk management principles are described in Chapter 3.

A process validation lifecycle model (US FDA Guidance for Industry: Process Validation [5], EMA Guideline on process validation for finished products – information and data to be provided in regulatory submissions [6]) can be applied to cleaning processes. Chapter 4 of this ISPE Guide explains the adaptation of a lifecycle model to cleaning validation.

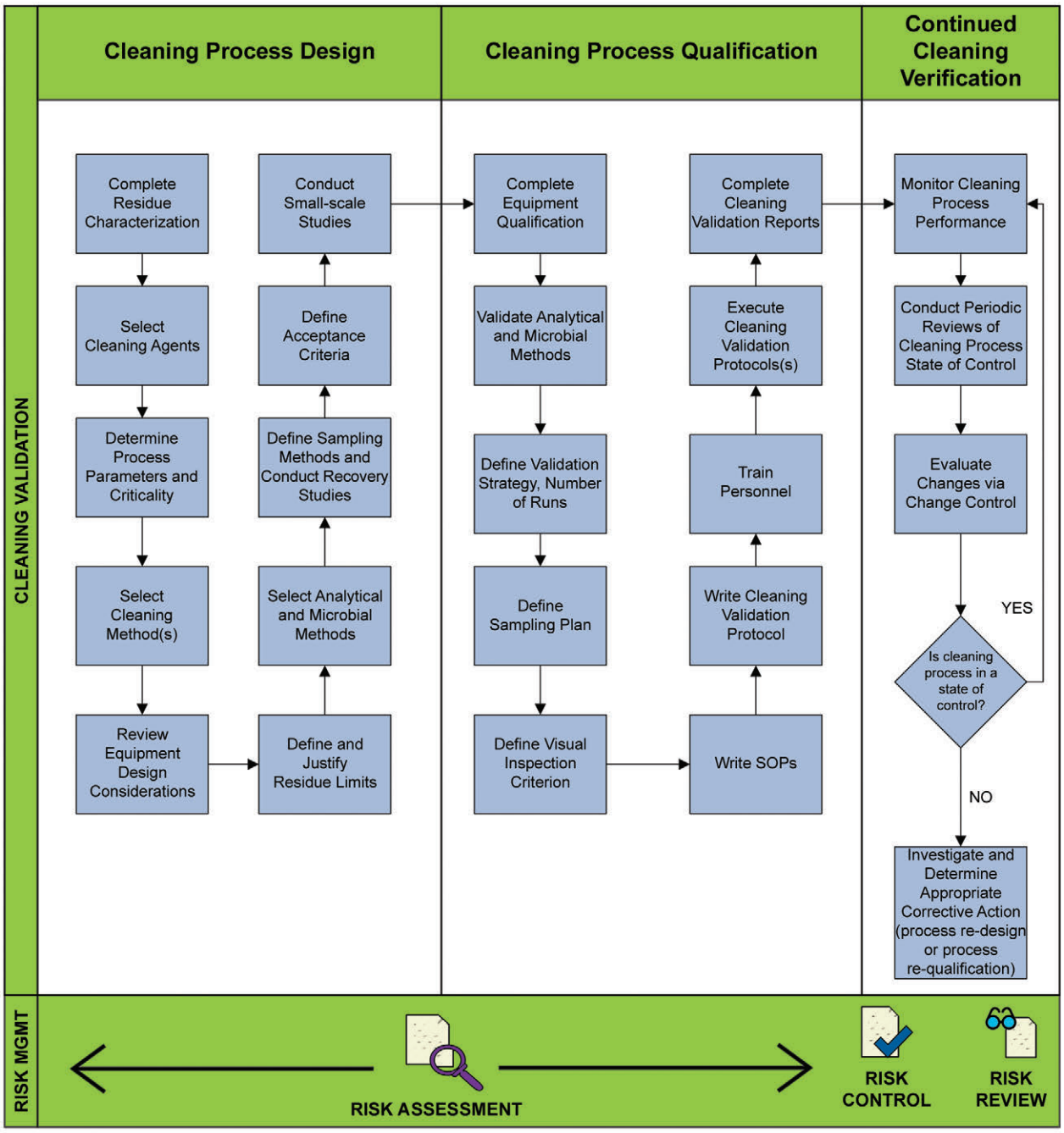
Cleaning validation strategies include the practice of justifying the grouping of equipment with the same design. Validating the cleaning method for multiple units of the same design can be simplified by selecting representative units for validation and an appropriate sampling plan following a science and risk-based approach. Similarly, validating a cleaning method using a minimum number of runs representing a combination of conditions with justification of the bracketing of parameters (e.g., size, volume, concentration) can also reduce the validation effort. Section 5.6 describes these practices in more detail.

The establishment of HBEL is the dominant approach for safety cleaning limits. When calculating the cleaning threshold values, the terms PDE, ADE, and HBEL are used interchangeably in this Guide.

Cleaning validation is not required for the manufacturing of clinical batches; cleaning verification is acceptable. Sometimes cleaning validation may not be practical, such as for infrequent batch runs; however, cleaning verification must be used until a process is validated. Refer to Section 9.7 for cleaning verification practices.

Figure 1.3 presents a roadmap to conduct cleaning validation, from process design to process qualification and continued process verification. This Guide provides help on how to approach each step of the cleaning process validation roadmap. Risk-based approaches are an integral part of the cleaning validation effort, and are also illustrated on the diagram, spanning from risk assessment to risk control and risk review.

Figure 1.3: Cleaning Process Validation Road Map



1.3.1 Current Considerations and Trends

Cleaning validation has been performed for several decades in the regulated industry. Despite the different approaches used to achieve cleanliness requirements, there are common areas that represent challenges for some manufacturers and should be taken into consideration when developing a comprehensive cleaning program.

Health-Based Exposure Limits (HBEL) – The establishment of cleaning limits using a HBEL approach is the recommended guidance for setting safe residue limits (see EMA [7]). However, there are several situations where alternate approaches for scientifically based limits are required. For example, actives manufactured from biological processes are usually inactivated, denatured, or degraded when strong cleaning processes are used. An appropriate HBEL may not be available to describe the safety risks of the resulting fragments or degradation products.[1] In addition, development of an Investigational Medicinal Product (IMP), or during early clinical batch production, may lack HBEL-specific information. Furthermore, legacy facilities may not use HBELs as part of their cleaning programs. The transition from traditional approaches to a HBEL-based cleaning program needs to be achieved. Refer to Sections 6.1.1.3 and 6.1.5 for guidance on how to transition from a legacy residue limit to a HBEL-based limit. See also Chapter 6 for additional guidance on how to justify limits for cleaning validation.

Corrosion Management – The corrosion of metal surfaces may reach a point where the established cleaning process is affected. Corrosion may also affect the formation of biofilms, as an increase in roughness may reduce the cleanability of the surface and promote biofilm formation. Each company should have a system to manage metal corrosion (e.g., rouge issues). Corrosion levels that can affect the performance of the validated cleaning process are those that release metal particles into the process, change the color of the rinse/swab samples, cause a failure of visual inspection, or change the equipment surface characteristics making it harder to clean. Monitoring vessel product contact surfaces and using visual inspection criteria to identify corrosion on surfaces should be part of the controls to manage this issue. Other preventive measures (e.g., periodic passivation or chemical treatments) should be considered for corrosion prone or critical equipment.

Furthermore, plastic surfaces may be impacted by chemical attack causing cleaning surface degradation, and glass surfaces may be subject to oxidative attack and delamination. Similar controls to the ones in place for corrosion management should be applied where necessary to ensure consistent and effective cleaning.

Manual Cleaning Processes – The reproducibility of manual cleaning processes represents a challenge to validate due to the variability of manual steps. Manual processes should be described in detail and designed in a robust way to ensure that the cleaning objectives are reached despite the variability in application. Operators should be trained and periodically assessed to prevent drifting of manual cleaning techniques or skills that may impact the final residue levels. Approaches such as alternating cleaning agents, extended cleaning times, or increased concentration of cleaning agents should be considered during the development of the cleaning process to improve process robustness. The reproducibility required for manual cleaning is more achievable with higher PDE/ADE products than with lower ones. It may not be possible to validate manual cleaning processes at the levels required for low PDE/ADE products, or at least extensive (and potentially prohibitive) work may be required. As such, consideration should be given to automated cleaning to allow greater consistency, or to dedicated equipment for these products. Refer to Appendix 6 for an example of parameter setting for manual cleaning procedures.

Buildup of Biological Residues – Clean in Place (CIP) systems may require occasional manual scrubbing of surfaces or the use of alternate cleaning agents to remove residues deposited from some biological processes. If left unattended, the residues may accumulate and affect the cleaning performance. Users should assess the risk of biological residue removal techniques and consider the formation of persistent residue deposits during cleaning process development, and ensure the process and frequency of residue removal is properly documented. Appropriate visual inspection criteria to determine the presence of biological residues should be included in the cleaning procedures of equipment impacted by these types of residues. Enzymatic degradation or manual cleaning steps should be considered in the cleaning process procedure [8].

Formation of Biofilm – Biofilms can be a persistent problem in GMP water systems, facilities, and some processes based on continuous manufacturing. Biofilm is the accumulation of microbial cells to a surface forming a film or layer of extracellular material. Prevention of biofilms, as well as their formation and removal from equipment surfaces, need to be considered during cleaning process development. Biocides and other sanitizing agents are used to prevent biofilm formation.

[1] *ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3] describes the approach of treating any degradation fragment as the product itself unless there is additional toxicological information to show differently.

Air/Liquid Interface in Vessels – Many processes leave residues at the air/liquid interface of vessels. If these residues are not removed, they will start to accumulate and affect the cleaning validation status of the equipment. Removal of air/liquid interface residues should be considered during the development of cleaning processes. These residues should also be removed manually when first detected, and until a validated effective cleaning process is implemented.

Maintenance Practices for Complex Systems – Highly automated systems require all components to work properly. System reliability becomes a challenge if individual components considered single points of failure start to fail (e.g., steam traps, gaskets use life). A risk assessment is recommended to evaluate which components should be emphasized in the maintenance reliability program and maintenance schedules (frequency of failure of components, useful life of gaskets in contact with product, etc.).

Drainability – Complex systems need to ensure full drainability of liquids after cleaning to ensure adequate Clean Hold Times (CHTs) and appropriate bioburden controls. Cleaned equipment intended for storage should be dried, usually accelerated by the use of Clean, Dry Air (CDA) (filtered air or nitrogen) after draining the system. Manual methods are commonly employed such as using a cloth or alcohol wipes, although these may be less reliable or not feasible for hard-to-reach surfaces.

Regulatory Harmonization and Mutual Recognition Agreements (MRA) – The developments in MRA between major regulatory agencies should drive toward greater harmonization of requirements and expectations. Cleaning validation can benefit from further alignment regarding the establishment of cleaning limits and overall management of a compliant cleaning program. During 2015, the PIC/S GMP Guide Annex 15 [9] was harmonized with EudraLex Annex 15 [4] to adopt HBEL as the basis for scientifically justified safe residue limits.

1.3.2 Key Terms

This section introduces key terms as they are used in the context of this Guide. Refer to Appendix 10 for an expanded listing of definitions.

Note: When calculating the cleaning threshold values, the terms PDE, ADE, and HBEL are used interchangeably in this Guide.

Acceptable Daily Exposure (ADE)

A dose that is unlikely to cause an adverse effect if an individual is exposed, by any route, at or below this dose every day for a lifetime [3].

Action Limit – *also known as* ***Action Level***

A parameter set by the user that, when exceeded, requires immediate intervention, including investigation of cause, and corrective action [10].

Alert Level – *also known as* ***Alert Limit***

A parameter set by the user that, when exceeded, gives an early warning of a drift from normal operational conditions, and should result in increased attention or corrective action [10].

Cleaning Agent

Chemical agent or solution used for cleaning. May be aqueous or solvent-based.

Detergent

A type of cleaning agent, usually aqueous-based and utilizing surfactants.

Health-Based Exposure Limits (HBEL)[2]

A daily dose or a substance below which no adverse effects are anticipated, by any route, even if exposure occurs for a lifetime. Derived from a structured scientific evaluation of relevant data [11].

Highly Hazardous Compounds

Compounds with low ADE/PDE values, for example, ≤ 10 µg/day. (See the *ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3] Section 5.2 for further explanation.)

Limit of Quantitation (LOQ) – *also known as* ***Quantitation Limit (QL)***

Lowest level of analyte that can be reliably measured with acceptable accuracy and precision.

Limit of Detection (LOD) – *also known as* ***Detection Limit (DL)***

Lowest level of analyte that can be detected but not necessarily quantified.

Maximum Allowable Carryover (MACO) *(also known as* ***MAC****)*

Calculated quantity of residue from a previous product when carried over into a different product that can represent potential harm to the patient.

With the introduction of safe cleaning limits based on HBELs, the MACO term should be considered a Maximum Safe Carryover (MSC), which is the maximum amount of carryover of a residual process residue (API, cleaning agent, degradant, and so forth) into the next product manufactured without presenting an appreciable health risk to patients [12]. For simplicity, this Guide has kept the terms MACO or Safe MACO to denote material carryovers from one product batch to the next product on shared equipment.

Nonhazardous Compounds

Compounds with high ADE/PDE values, for example, ≥ 100 µg/day.

Permitted Daily Exposure (PDE)

A substance-specific dose that is unlikely to cause an adverse effect if an individual is exposed at or below this dose every day for a lifetime [11].

Safety Limit (SL) – *also known as* ***Acceptable Residue Limit (ARL)***

Represents the acceptable cleaning limit based on HBELs corresponding to a safe amount of residue in the next product dose (i.e., DP) or batch (i.e., DS)

[2] Establishing a HBEL involves the identification of hazard conditions (toxicity), evaluating the therapeutic or adverse effects, determining NOAEL (mg/kg/day), establishing a PDE or ADE, and calculating a MACO [11].

2 Cleaning Regulations

Regulatory agencies have provided guidance documents describing the expectations for cleaning to prevent contamination or adulteration of products. These guidance documents focus on requirements to ensure control of the cleaning process.

While regulations vary between countries, they are aligned in the general principles to confirm that pharmaceutical products are safe, efficacious, and free from adulteration by other components or contaminants. Table 2.1 contains frequently used guidance documents that describe expectations for compliant cleaning processes.

Table 2.1: Regulatory Guidances Related to Cleaning Validation

Regulatory Authority or Organization	Guidance
Canada	Cleaning Validation Guidelines GUIDE-0028 (2008) Canada Health Products and Food Branch Inspectorate Guidance Document [13]
China	GMP Annex 1: Sterile Medicinal Products, revised 2010 [14]
EMA (EU)	Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities, November 2014 [11]
	EudraLex Volume 4 – Good Manufacturing Practice (GMP) guidelines, Chapter 5 – Production, March 2015 [15]
	EudraLex Volume 4, Good Manufacturing Practice (GMP) guidelines, Annex 15: Qualification and Validation, October 2015 [4]
	Questions and answers on implementation of risk-based prevention of cross-contamination in production and 'Guideline on setting health-based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities,' April 2018 [7]
FDA (US)	21 CFR 211 (specifically 211.63, 211.65, 211.67, and 211.113) [16]
	Guide to Inspection of Validation of Cleaning Processes, 1993 [17]
	Pharmaceutical CGMPs for the 21th Century – A Risk-Based Approach, Final Report, 2004 [18]
	Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice, September 2004 [19]
	Guidance for Industry: Process Validation: General Principles and Practices, January 2011 [5]
	Guidance for Industry: Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients, Questions and Answers, April 2018 [20]
ICH	Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients, November 2000 [21]
	Q9 Quality Risk Management, November 2009 [22]

Table 2.1: Regulatory Guidances Related to Cleaning Validation (continued)

Regulatory Authority or Organization	Guidance
PIC/S	Validation Master Plan, Installation and Operational Qualification, Non-Sterile Process Validation, Cleaning Validation, PI 006-3 September 2007 [23]
	Guide to Good Manufacturing Practice for Medical Products, Annex 15, Qualification and Validation, 2015 [9]
	Aide Memoire: Cross Contamination in Shared Facilities, PI 043-1, July 2018 [24]
	Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities, PI 046-1, July 2018 [25]
	Aide Memoire: Inspection of Health Based Exposure Limit (HBEL) Assessments and Use in Quality Risk Management, PI 052-1, June 2020 [26]
	Questions and Answers on Implementation of Risk-Based Prevention of Cross-Contamination in Production and 'Guideline on Setting Health-Based Exposure Limits for Use in Risk Identification in the Manufacture of Different Medicinal Products in Shared Facilities', PI 053-1, June 2020 [27]
WHO	Technical Report Series, No. 957, Annex 2 WHO good manufacturing practices for active pharmaceutical ingredients, 2010 [28]
	Draft Working document QAS/20.849, Points to consider on the different approaches – including HBEL – to establish carryover limits in cleaning validation for identification of contamination risks when manufacturing in shared facilities, May 2020 [29]

In addition to these regulatory guidance documents, there are several industry guides, technical reports, and standards relevant to cleaning and cleaning validation such as those shown in Table 2.2.

Table 2.2: Cleaning and Cleaning Validation Industry References

3-A Sanitary Standards, Inc. (3-A SSI)] [30]
APIC Guidance on Aspects of Cleaning Validation in Active Pharmaceutical Ingredient Plants (2016) [31]
ASME BPE - 2019 Bioprocessing Equipment [32]
ASTM E3106-18e1 Standard Guide for Science-Based and Risk-Based Cleaning Process Development and Validation (2018) [33]
ASTM E3219-20 Standard Guide for Derivation of Health-Based Exposure Limits (HBELs) (2020) [12]
European Hygienic Engineering & Design Group (EHEDG) [34]
ISO 13408-4:2005 Aseptic processing of health care products – Part 4: Clean-in-place technologies [35]
ISPE Baseline® Guide – Volume 7: Risk-Based Manufacture of Pharmaceutical Products [Risk-MaPP] (Second Edition) (2017) [3]
PDA Technical Report No. 14, Validation of Column-Based Chromatography Processes for the Purification of Proteins (2008) [36]
PDA Technical Report No. 29, Points to Consider for Cleaning Validation (2012) [37]
PDA Technical Report No. 49, Points to Consider for Biotechnology Cleaning Validation (2010) [38]

3 Risk Management

3.1 Risk Management Description Overview and Regulatory Expectations

Quality Risk Management (QRM) is a rational approach to enable good decisions. Cleaning validation, like other GMP validation activities, is not exempted from the regulatory expectation of using risk management to control potential hazards, reduce risks, and establish sound cleaning processes. In fact, a risk-based cleaning validation strategy with justifiable and achievable acceptance criteria is crucial in attaining a compliant cleaning validation program.

For example, validation master plans for cleaning should either start with a documented risk assessment exercise or at least include the risk assessment process in the early stages of developing a new program. In today's pharmaceutical regulatory landscape, a retrospective risk assessment is not only a good practice, it is expected within cleaning programs, even if that program has been grandfathered as acceptable.

3.1.1 Regulatory Expectations

Quality risk management is a regulatory expectation, as noted in FDA's Pharmaceutical CGMPs for the 21th Century [18], which aims to encourage implementation of risk-based approaches that focus on critical areas for maintaining or improving product quality.

Expectations for the application of QRM principles to validation are clearly stated in regulatory documents.

For example, EudraLex Annex 15 [4] states applications for QRM in validation:

- To determine scope and extent of qualification and validation.
- To reassess risks after gaining more knowledge from commercial production.
- To determine criticality of process parameters.
- To evaluate planned changes to determine potential impact.
- To justify bracketing approaches.
- To determine the variable factors which influence cleaning effectiveness and performance.
- To justify selected cleaning limits.
- To justify the number of times the cleaning procedure should be executed (number of runs) for validation.
- To determine the risks presented by microbial and endotoxin contamination during the development of cleaning validation protocols.

Similarly, the FDA Guidance for Industry: Process Validation [5] calls for QRM principles to be applied:

- To determine the degree of controls needed to control process variation.
- To screen potential variables for Design of Experiment (DOE) studies to minimize the total number of experiments conducted while maximizing knowledge gained.
- To support the prioritization of certain equipment qualification activities and to identify the level of effort needed in both the performance and documentation of qualification activities.

- To justify the sampling location, sampling frequency, and confidence level for sampling plans.
- To determine criteria for process performance indicators.

Furthermore, EMA in its guidance for industry to set HBELs recognizes that cleaning is a risk reducing measure.

> *"A more scientific case by case approach is warranted for risk identification and to support risk reduction measures for all classes of pharmaceutical substances."* [11]

In addition, once a health-based assessment has been completed, the data is used as an input to a comprehensive risk management process to identify risks, determine controls necessary to address the risks, and evaluate if existing technical and organizational controls are adequate, or need to be supplemented with additional controls.

> *"It is expected that for products which present a higher potential harm to patients/animals, more elaborate organisational and technical control measures will be required. Using a structured Quality Risk Management process, manufacturers should consider the risks of cross contamination down to the established level from the HBEL. During the QRM study manufacturers should consider how easily such a quantity of contamination could occur, without detection, at batch and unit dose level."* [7]

The cleaning validation element of cross contamination control should be guided first by the HBEL and then consider the impact or contribution from other factors that influence the cleaning and requirements to ensure consistency and robustness in validation.

These expectations clearly demonstrate that risk management should be an integral part of cleaning validation efforts.

The premise that a company's quality system should include a formal risk management program further drives the need for cleaning validation to be approved by Quality Assurance (QA). It should have a foundation in observing, remediating, and controlling the risks inherent to any equipment that has been cleaned and ready for release for GMP use.

3.1.2 Risk Management Models

ICH Q9 [22] outlines a risk management process that is iterative and consists of the identification of hazards, and the analysis and evaluation of risks associated with exposure to those hazards, as well as control strategies to manage the risks. It has three distinct phases for the application of risk management: Risk Assessment, Risk Control, and Risk Review.

1. Risk assessment involves the identification of risks (hazards and their impact), the analysis of the risks, and the evaluation and prioritization of risks.
2. Risk control includes decision making to reduce/accept risks, create a control strategy, and communicate to stakeholders.
3. Risk review monitors the results of system performance and changes on a periodic basis, initiating additional risks assessments if needed.

During all phases, the output of each phase is communicated to stakeholders for appropriate knowledge management and decision making, especially after completing the Risk Control phase, where a control strategy is defined [22].

The risk management system should always be driven with a focus on the patient first, yet should not exclude potential impacts to the business, as the process must be considered sustainable with empirical data to support its claims.

Figure 3.1 adapts the ICH Q9 [22] model to cleaning risk management, linking cleaning validation activities with risk management principles.

- **Risk Identification:** Hazards from the environment, equipment, methods, chemical and microbial entities, and personnel are identified. The hazards may have an impact on the final residues after cleaning and therefore are considered risks.

- **Risk Analysis:** These risks are further analyzed to achieve a better understanding of the process and to prioritize their impact to cleaning. During this phase, process knowledge is increased by design reviews, data review, and studies to understand interactions between process parameters, equipment, environment, and personnel.

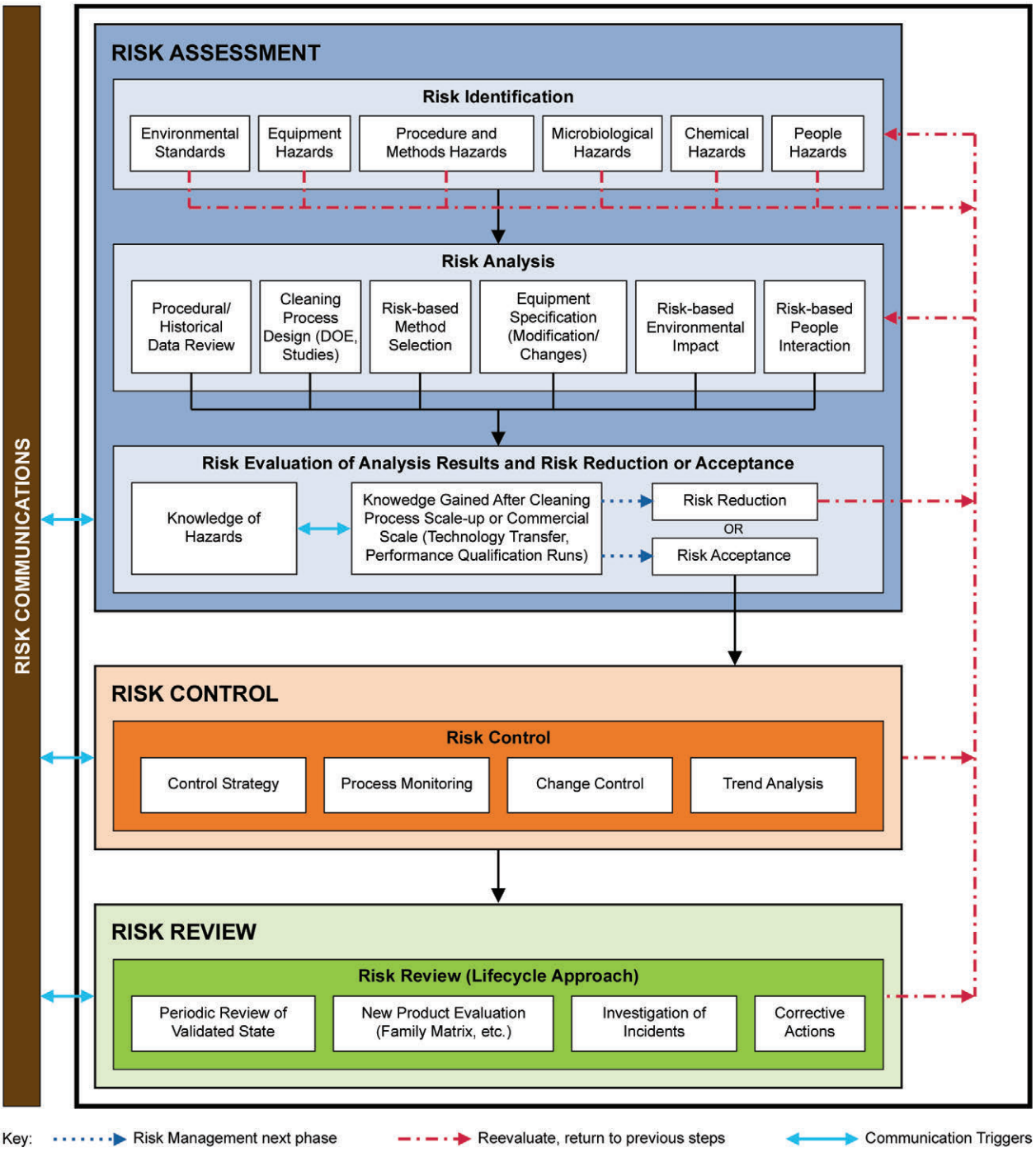
- **Risk Evaluation:** Risks are evaluated, and additional controls are identified to mitigate the risks (risk reduction) or accept the risk (risk acceptance).

- **Risk Control:** During risk control, final decisions are made regarding acceptable risks, and a control strategy is completed to ensure mitigating design, procedural, and technical controls are applied and remain in place. This control strategy is communicated formally to stakeholders.

- **Risk Review:** Risks are reviewed periodically or when significant or new hazards are introduced, such as the introduction of new products and after major incidents or events.

There is a relationship between QRM principles and the different phases of the validation lifecycle. As illustrated in Chapter 1, Figure 1.3, risk management is present throughout the validation lifecycle.

Figure 3.1: Cleaning Risk Management Process



There are many tools available to support the application of QRM principles. The most common are:

- Failure Mode Effects Analysis (FMEA)
- Fault Tree Analysis (FTA)
- Hazzard Analysis and Critical Control Points (HACCP)

- Fishbone/Ishikawa

One hint to secure support between Subject Matter Experts (SMEs) and colleagues during risk assessments is to simplify (when possible) the logical path from supporting data to the outcome of the impact assessment. In-depth information on other risk assessment tools can be found in focused guidance from ISPE [3] or other reputable sources that give a full view of the tools for QRM use [39].

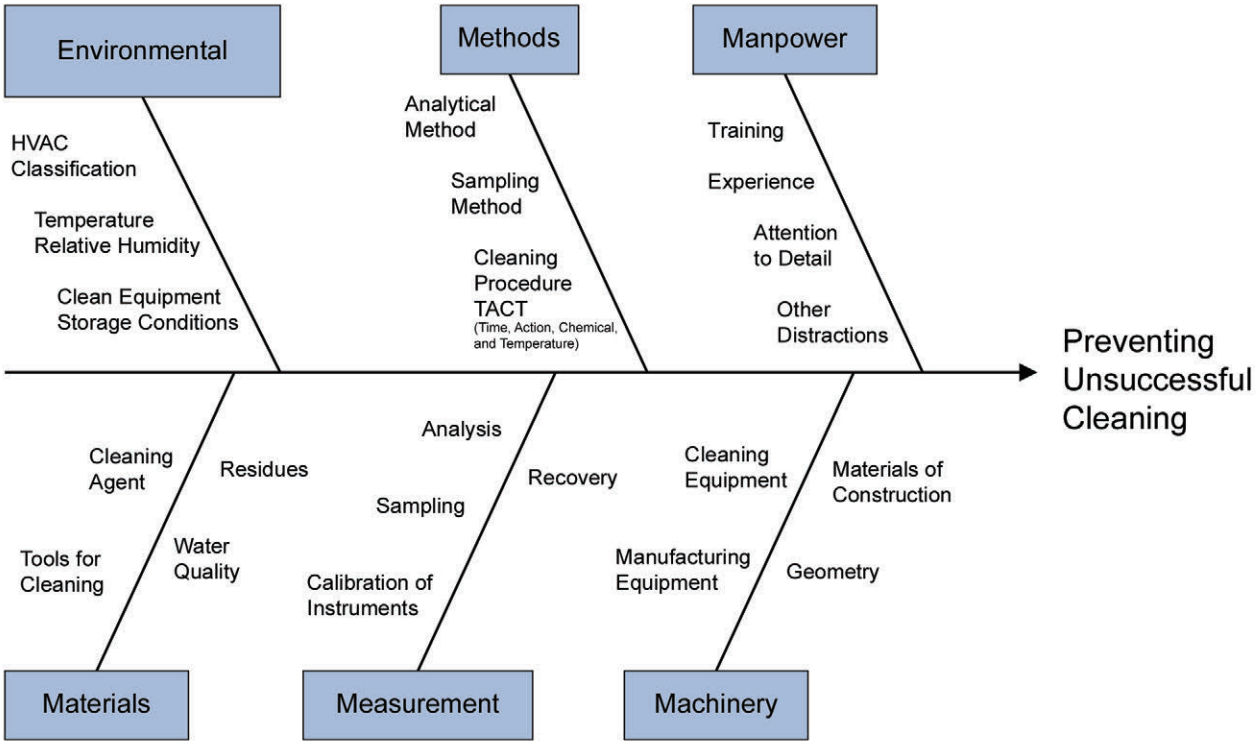
3.1.3 Sources of Variation

Unsuccessful cleaning of manufacturing equipment, including major and minor/ancillary equipment and change parts, as well as utensils, presents a risk to product quality and could ultimately impact patient safety if not adequately addressed.

One approach to take when determining causes of unsuccessful cleaning during or post cleaning validation is to start with an outline of the characteristics within the cleaning practices for risk assessment. The outline should identify the equipment, cleaning agents, Active Pharmaceutical Ingredients (APIs), types of products produced, microbial particulates/bioburden, lubricants, and procedures allocated to control the operational and cleaning processes. The cleaning procedures themselves can be a source of risk, especially manual procedures that have not been assessed in this manner; thus, a formal discussion on these materials and practices should be thoroughly documented.

The fishbone/Ishikawa diagram in Figure 3.2 provides an example[3] of the sources of variation that could result in unsuccessful cleaning. From the fishbone outline, various impact assessment challenges can be derived using risk-based scenarios where failure could occur.

Figure 3.2: Potential Sources of Variation Related to Cleaning
Adapted from ISPE Training Slides [40]



[3] The example provided herein is for illustrative purposes and is in no way meant to imply that this is the only way to perform the risk assessment process or risk identification task. Additionally, it is not intended to be an exhaustive, all-inclusive example.

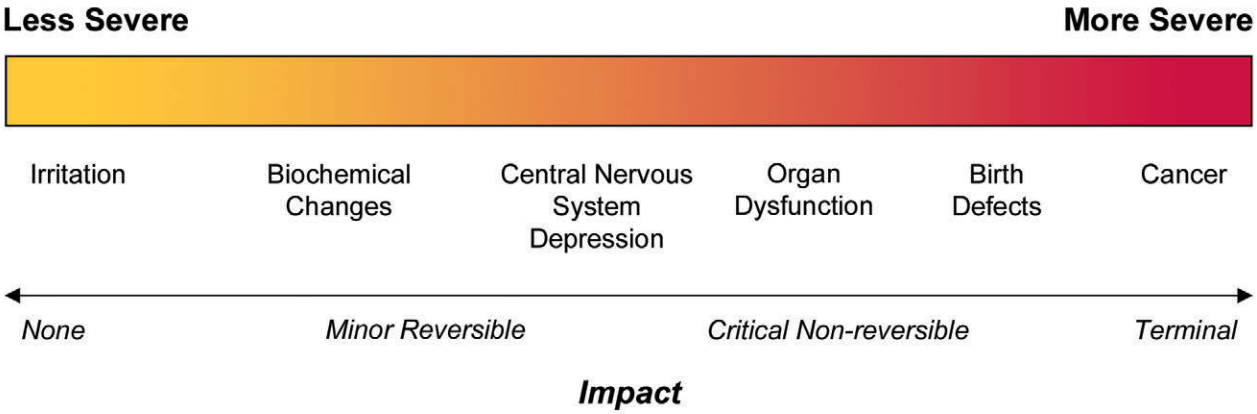
3.2 Risk Management Applied to the Cleaning Validation Program

Risk management for cleaning processes focuses on the hazard represented by the active and chemical residues on product contact surfaces, the impact or severity represented by such residues, the likelihood of these residues being present, and the ability to detect them.

Many cleaning regimens originally focused on visual cleanliness as a measure of success. However, there are multiple sources of hazards in a cleaning process that need to be well understood in order to guide the planning and decision making for developing effective and compliant cleaning processes.

One of the most important hazards in a cleaning program comes from the type of soils to be cleaned. Some chemicals and actives present low risks to patients and others higher risks, as represented in Figure 3.3.

Figure 3.3: Hazard Continuum [3]



The recommended approach for protecting patients from chemical and active residue is to use toxicological data to determine the residue HBELs, and then use the results to set cleaning limits. This approach is strongly encouraged by the EMA: Questions and answers on implementation of risk-based prevention of cross-contamination in production and 'Guideline on setting health-based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities' [7], Question 3:

> *"Once the health-based assessment has been completed and the HBEL confirmed, these data should be used via a Quality Risk Management process to determine what controls need to be put in place and to assess if existing organisational and technical control measures are adequate or if they need to be supplemented. This Quality Risk Management process should be carried out prospectively in the case of new equipment/facility to determine what control measures are required."*

Additionally, organizations need to implement appropriate technical and procedural controls commensurate to the risks for contamination. The QRM process should consider cross contamination risks down to the established HBEL and evaluate how the risks may impact a batch or unit dose [7].

Table 3.1 lists a summary of specific applications of QRM to cleaning validation.

Table 3.1: Examples of QRM Applications in Cleaning Validation

Cleaning Validation Milestone	Tasks	Considerations
Validation Master Plan	Matrix approach or testing all products (multiproduct facility)	Define "worst-case" approach for validating products and API
	Define sampling plan	Sampling methods, locations, frequency, and confidence levels
	Selection of analytical methods	HPLC versus Total Organic Carbon (TOC) along with appropriate microbial testing techniques, LOD, method variability
Design and Development	Determine target HBEL and perform risk assessment to evaluate cleaning limits	Availability of PDE/ADE or clinical data Impact to existing control plans
	Determine parameters for the cleaning process Justify bracketing and grouping approaches	Determine interactions between parameters and justify best ranges for effectiveness and performance
Qualification (PPQ)	Test equipment hard to clean areas to confirm CV	Confirm equipment is sufficiently clean to complete CV through appropriate testing and sampling locations
	Determine number of runs for cleaning qualification	Optimum number of times cleaning procedures must be executed to cover the scope of cleaning validation
Verification (CPV)	Find areas where the processes can be improved	CHT studies or reduced testing strategies during campaigning production, evaluation of current hazards to update controls
Ongoing Monitoring	Ensure that cleaning limits remain appropriate and that periodic testing satisfies requirements	Additional process knowledge could change initial PDE/ADE calculations; verification via spot checking the current hazards can reduce risk
	Evaluate opportunities for reduced testing	Level of controls (testing) should be commensurate to current level of risks
Periodic Review	Assemble a cross-functional team for assessment to evaluate and report on the cleaning validation program effectiveness	This report should be available for regulatory review to highlight updates in cleaning validation control strategies
Change Management	Evaluate planned changes to determine potential impact	Consider impact to validation control plan. Consider accumulative impact of changes.

Additional risk evaluation applications are discussed in Sections 3.2.1 to 3.2.4 using the cleaning validation lifecycle.

3.2.1 Initial Cleaning Validation Assessments

Validation assessments at this stage help determine the scope and extent of qualification and validation efforts from a perspective of risk.

The assessment conclusion should recommend validation, verification (i.e., dedicated indirect equipment), or no validation (i.e., waste collection vessels) requirements. The assessment should also identify the extent of the effort (number of runs or products involved, studies anticipated to be needed, special training considerations, etc.). Table 3.2 provides a list of areas to consider during assessments for validation.

Table 3.2: Validation Assessment Elements for Cleaning

Scope	Evaluation Scope
Analytical Methods	• Supplies (e.g., containers, swabs) • Specificity • Robustness • Range • Sensitivity • Active degradation • Procedure for unknown peaks
Cleaning Agents	• ADE/PDE levels • Cleaning effectiveness • Rinsability • Detectability
Cleaning Cycle Development	• Number of runs
Cleaning Procedure	• TACT* characteristics • Reproducibility (e.g., automated, semiautomated, manual) • Level of training • Level of detail (e.g., manual cleaning)
Cleaning Qualification Execution	• Number of runs
Hold Times	• Dirty (e.g., effect on cleanability) • Clean (e.g., length, storage, proliferation)
Manufacturing Equipment	• Equipment and process product contact points • Equipment complexity (e.g., components, circuits, shape) • Buildup concerns • Grouping (e.g., products, equipment)
Manufacturing Process	• Soil cleanability • Process step criticality • Soil load/batch size variation
Residue	• ADE/PDE levels of process residues (e.g., APIs, excipients, degradants, process aides, lubricants) • Cleanability • Detectability
Safety Cleaning Limits	• Cleaning limit calculation and justification • Rationale for chosen cleaning limit method • HBEL safety limit calculation and justification
Sampling Approach	• Indirect (e.g., timing, sequence (in-line, grab sample, separate)) • Direct (e.g., accessibility, locations) • Visual inspection (e.g., accessibility, lighting)
Sampling Recovery	• Recoverability (cleaning agent, active ingredient, and microbial) • Materials of construction • Percent recovery
*TACT = Time, Action, Chemical, and Temperature	

3.2.2 Introduction of New Products Risk Assessments

Introducing new products into a facility requires in-depth knowledge of the new hazards this may imply. When assessing the validation effort for legacy cleaning processes, the assessment should consider factors such as:

- Historical knowledge of current processes

- Process and equipment similarities
- Cleaning methods used
- Cleaning agents used
- Equivalent or worst-case excipients/formulations

If the new product to be introduced does not represent a new worst-case chemical or active to clean, then the existing cleaning process may be considered sufficient. A comprehensive risk assessment is completed to evaluate any changes to the existing control plans, including updating justification reports for HBEL, sampling, and all other elements of the cleaning program. Conversely, if the new product introduces new chemicals or actives that exceed the capability of the current cleaning process, then a comprehensive evaluation of the cleaning program is required.

Refer to Appendix 8 for a case study applying QRM principles to the introduction of new products in an existing facility.

3.2.3 Ongoing Monitoring Maintenance Risk Assessments

The level, type, and frequency of testing during the ongoing monitoring of a validated cleaning process is evaluated via an initial risk assessment. This ensures the implementation of a monitoring program that is science and risk-based. Additionally, as risk is reduced, the level of monitoring can be reduced.

The scope of the risk assessment should be determined early in the process, ideally as part of the Validation Master Plan (VMP) and periodic review of cleaning processes.

3.2.4 Routine Operation of Cleaning Process Risk Assessments

As part of the ongoing verification of cleaning processes, a risk assessment is performed to assess the cleaning process for changes in hazards and corresponding risks. It assesses changes in processes, procedures, activities, and determines points of highest impact. The assessment should be a living document to be updated on a periodic schedule (i.e., during cleaning periodic reviews) or be part of a control strategy to capture changes to the process.

HACCP or modified versions are good for assessing the process and determining potential failure points or weak points within the process.

Considerations:

- Changes documented as part of the cleaning process periodic review
- In-process sampling methods
- Process performance
- Changes in manufacturing process steps
- Changeover procedures
- Training program
- Changes in CIP/COP recipes and alarms
- New product and equipment grouping
- Equipment maintenance

3.2.5 *Additional Applications*

For complex processes or scenarios, separate risk assessments can be used to evaluate special hazards and identify appropriate control measures to mitigate risks. For example:

- Implementing an aggressive cleaning validation grouping strategy
- Using reduced Material of Construction (MOC) recovery groupings to represent larger groups of materials
- Optimizing the level of detail for non-critical manual cleaning activities

These scenarios require that SMEs understand how to effectively analyze and control potential hazards. The output of these risk assessments is used to support better decisions.

3.3 Points to Consider When Using QRM Tools for Cleaning Programs

The risk evaluation approaches in this section can be tailored for the initial cleaning validation process, a new product introduction (i.e., legacy process), or maintenance of the cleaning program (i.e., CPV and periodic review). They are presented as an example for illustrative purposes and are not meant to be an exhaustive list. Each company applies the appropriate considerations for risk evaluations aligned with their risk procedures and hazards. Comprehensive applications of these concepts are found in the *ISPE Baseline® Guide – Volume 7: Risk-Based Manufacture of Pharmaceutical Products [Risk-MaPP] (Second Edition)* [3].

3.3.1 *Severity*

When evaluating severity, a decision must be made to assess toxicological severity (i.e., low ADE/PDE values (high hazard), biological, topical) from an industry or site perspective. It is recommended to assess risk from a patient perspective. Table 3.3 presents a list of considerations when assessing severity.

Table 3.3: Assessing Severity – Topics to Consider

Severity	Examples of Rating Factors				
	5	4	3	2	1
Equipment Use	Shared				Dedicated
Direct or Indirect	Product		Excipient		Buffers
Type of Product	Low ADE/PDE (high hazard)	Rx (Prescription)	Over the Counter (OTC)	Nutritional	Cosmetics
HBEL	≤ 1 µg/day		≤ 10 µg/day		≥ 100 µg/day
Stability of Active	Stable	25% deactivated/ degraded	50% deactivated/ degraded	75% deactivated/ degraded	Completely deactivated/ degraded
Route of Administration	Intravenous		Oral		Topical
Cleaning Agents	Formulated		Commodity		Water only
Microbial Concerns	Dry manufacturing processing step	Dry processing	Bacteriostatic or low-water activity Process material	Sanitization steps prior to use	Sterilization steps prior to use
Proximity to Patient or Further Purification Steps	Filling*	Formulation*	Final Purification	Initial Purification or Recovery	Fermentation
*For drug product facilities, the risk factors could be 5 and 1 for filling or tableting, respectively. 5 = High Severity / 1 = Low Severity					

3.3.2 Probability

"What is the likelihood (probability) it will go wrong?" [3]

Table 3.4 provides a list of considerations when assessing probability.

Table 3.4 Assessing Probability – Topics to Consider

Probability	Examples of Rating Factors				
	5	4	3	2	1
Soil Type	Pastes		Solids		Liquids
Cleanability (Due to Solubility or Manufacturing Process)	Difficult to clean		Moderately hard to clean		Easy to clean
Cleaning Reproducibility	Manual		Semiautomated		Automated
Equipment Design	Complex geometry and high number of internal components	Simple geometry and high number of internal components	Complex geometry with low number of internal components	Simple geometry with low number of internal components	Simple geometry with no internal components, except valve
Microbial Load	Ambient process water (WFI or PW) cleaning solutions	Bacteriostatic or low-water activity Process material	Hot process water (WFI or PW) cleaning solutions	Caustic and/or acidic cleaning solutions	Steam in Place (SIP) or sanitization steps prior to use
Deviations	Cleaning failures due to cleaning process failure		Cleaning failures due to system failures		No known cleaning failures
Age of Cleaning Validation	Over 20 years	16 to 20 years	11 to 15 years	6 to 10 years	Under 5 years
CV Results (Worst Case)	Above 51%	21%–50%	10%–20% of limit	Below LOQ	Below LOD
Historical Routine Monitoring Trending Data	Historically outside of sigma		Variable trending history		Consistent trending within sigma
5 = Higher Likelihood / 1 = Lower Likelihood					

3.3.3 Risk Prioritization[4]

There are many approaches to prioritizing risks. It is critical to establish definitions and ranges for risk potentials. Risk Priority Numbers (RPN) can be derived from a matrix or algorithm that takes into consideration the likeliness, detection, and impact of risks to produce a scalable value. Once the risks are prioritized, a mitigation strategy can be agreed to. Table 3.5 presents an FMEA scoring table as an example to help determine relative priorities of risks.

Table 3.5: Sample FMEA Scoring [3]

RPN Value	Severity	Occurrence	Detection
10	Injury to a patient or employee; ADE < 1 µg/day	More than once per batch	Not detectable by current methods
7	Cause extreme customer dissatisfaction; ADE = 1 > 10 µg/day	Once per batch	All manually inspected
5	Something likely to result in a complaint; ADE = 10 > 100 µg/day	Once per six months	Statistical sampling
3	Minor nuisance resulting in no loss; ADE = 100 > 1000 µg/day	Once every one to three years	100% inspection
1	Be unnoticed and not affect performance; ADE ≥ 1000 µg/day	One occurrence in greater than five years	Obvious or controlled and monitored and alarmed by control system

[4] A core element of the FMEA tool is to use risk scores. Scoring is arbitrary and as such extreme caution should be applied when scoring is used as a decision-making process for risk mitigation. Approaches to set risk scores (e.g., RPN) should be defined by qualified personnel and supported by a sound, consistent, and thoroughly documented process.

4 Cleaning Validation Principles

4.1 Cleaning Validation Lifecycle

Cleaning is considered a critical process in the manufacturing of pharmaceutical products [41]. A traditional cleaning validation program places emphasis on demonstrating that the cleaning method works as intended through a qualification program. However, current practices recognize that a better approach is to treat cleaning validation as a lifecycle, where the emphasis is shifted from performing cleaning qualifications to performing cleaning development and ongoing cleaning verification during the use of the cleaning method.

The cleaning validation lifecycle follows the model defined by FDA [5], the concepts defined in the EMA Process Validation Guideline [6], and other regulatory bodies for process validation.

> *"Although the rigor of process design and the timing of implementation for some 'stages' may differ, the science and risk-based methodology described in the FDA guidance is equally applicable to cleaning processes."* [3]

For existing operations and processes, the introduction of new products or residues may require an assessment of the suitability of existing cleaning method designs, rather than forcing a new design of cleaning methods.

The main elements of the FDA lifecycle model [5] are illustrated in Figure 4.1. The lifecycle model adopts the principles of Quality by Design, which states that quality cannot be assessed by testing alone. Therefore, processes need to be developed, critical parameters need to be identified, and the cleaning process monitored to verify ongoing performance and ensure quality output. The quality output expected from a cleaning process is the achievement of consistent cleaning of manufacturing product contact surfaces at a target cleanliness level that represents no harm to patients or impact to product quality (safety, identity, strength, potency).

The lifecycle approach for cleaning validation is more comprehensive than the traditional approach as application of the lifecycle ensures that the cleaning process remains in a state of control and provides a logical progression for gaining knowledge for process improvements. A comparison between the approaches is summarized in Table 4.1.

Implementing a lifecycle approach can be challenging for legacy products where the development of the cleaning process may not be fully documented. However, a company can benefit from understanding the cleaning process parameters and design constraints when dealing with cleaning method changes or investigating failures. Better knowledge of the cleaning process provides the tools necessary to assess potential manufacturing cross-contamination risks and helps to ensure a compliant and effective cleaning program.

Figure 4.1: FDA Model of Process Validation Lifecycle
Adapted from the FDA Presentation "Process Validation, A Lifecycle Approach" [42]

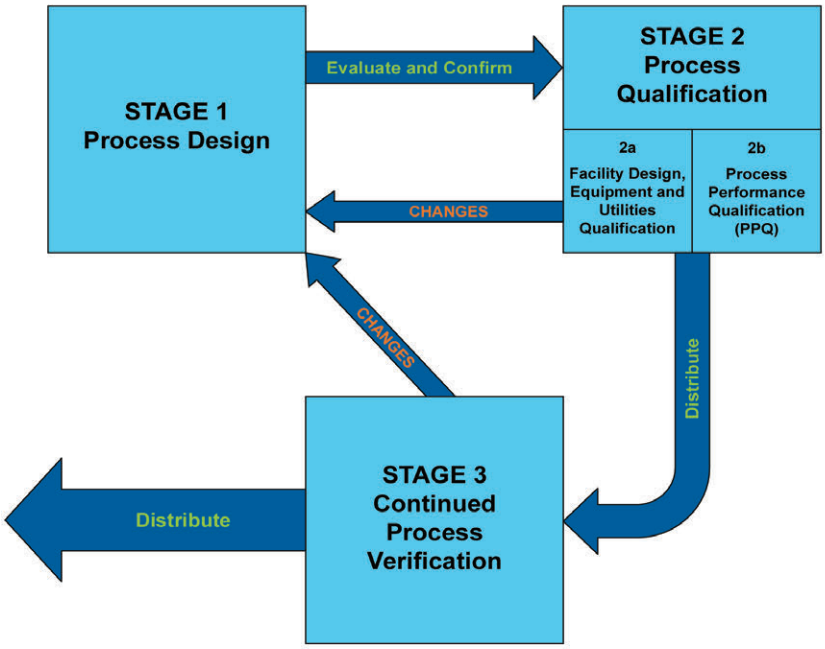


Table 4.1: Comparison Between a Traditional Approach and a Lifecycle Approach to Cleaning Validation

Traditional Approach	Lifecycle Approach
• Identify APIs and chemicals to clean • Select and qualify cleaning agents • Create Standard Operating Procedures (SOPs) for cleaning equipment • Determine cleaning procedure parameters • Establish sampling plan (sampling locations and methods) • Select validated analytical methods • Complete recovery studies • Develop acceptance criteria • Select equipment groups or families to simplify validation • Determine number of runs for validation (minimum of 3 runs considered acceptable) • Write validation protocol • Qualify equipment and methods • Train personnel • Execute cleaning validation protocol • Maintain ongoing control via change management • Monitor cleaning periodically	• Stage 1 – Cleaning Process Design: Typical activities include creating a cleaning validation plan, selecting cleaning agents, determining Health-Based Exposure Limits (HBEL), defining critical parameters following Quality Risk Management principles, characterizing residues, evaluating parameter interactions, completing recovery studies, selecting validated analytical methods, reviewing equipment design, grouping equipment, defining limits and acceptance criteria. • Stage 2 – Cleaning Process Qualification: Typical activities include conducting next phases of the validation plan, qualifying equipment, reviewing utilities readiness, qualifying suppliers, selecting sampling site, justifying number of qualification runs, creating a cleaning qualification protocol, training personnel, executing the cleaning qualification protocol, and issuing the final validation report. Knowledge gained during this stage may require going back to Stage 1 for further development. Otherwise, proceed to Stage 3. • Stage 3 – Continued Cleaning Verification (Ongoing Cleaning Verification): Typical activities include establishing periodic reviews, determining extension of PV sampling and testing, monitoring process capabilities, reviewing deviations and changes. Knowledge gained during this stage may require going back to Stage 1 for further development or for revalidation at Stage 2.

It is possible to introduce changes to previous stages in the lifecycle model based on knowledge gained during the execution of each stage. Risk-based approaches and assessments are conducted throughout the lifecycle, especially during Stage 1 and Stage 2 of the model.

Application of the Process Lifecycle Model to Cleaning Validation

The process lifecycle approach is a way to standardize a company's manufacturing and cleaning process [41]. There are three stages defined in the FDA process validation approach [5]: Stage 1: Process Design, Stage 2: Process Qualification, and Stage 3: Continued Process Verification. Similar terminology can be found in other regulatory guidances (see Table 4.2). The following analogous terms are defined for the cleaning process lifecycle:

- Stage 1: Cleaning Process Design – The cleaning process is defined based on knowledge gained through development and scale-up activities.
- Stage 2: Cleaning Process Performance Qualification (Cleaning PPQ) – The cleaning process is executed to demonstrate that the process, as developed and designed, produces the expected results in a reproducible manner.
- Stage 3: Continued Cleaning Process Verification – Ensures that critical cleaning process variables are monitored and that the process operates in a state of control.

A Cleaning Validation Master Plan (CVMP) can be created as early as in Stage 1 to cover design aspects of cleaning validation. The CVMP outlines the principles involved in the qualification of cleaning equipment, utilities, systems, and validation of cleaning processes. The CVMP also describes the written program to achieve and maintain a validated cleaning process. The information for a CVMP can also reside in an overarching facility VMP. Refer to Section 4.3 for additional details.

Table 4.2 compares the process validation regulatory stages with the terminology used in this Guide.

Table 4.2: Goals and Typical Activities of the Stages of Process Validation and Cleaning Validation
Adapted from Table 2.1 in ISPE Good Practice Guide: Practical Application of the Lifecycle Approach to Process Validation [43]

Stage of Validation	Process Validation Terminology	Goals	Cleaning Validation Lifecycle Approach Terminology Used in this Guide
Stage 1	Process Design (FDA/WHO) [5, 44] Pharmaceutical Development (EU) [45]	Define and design process	Cleaning Process Design
Stage 2	Process Qualification (FDA/WHO) [5, 44] Process Validation (EU/PIC/S) [4, 9]	Process is executed to demonstrate the process is capable of reproducible commercial manufacturing Cleaning process is executed to demonstrate that the process produces the expected results in a reproducible manner	Cleaning Process Performance Qualification (Cleaning PPQ)
Stage 3	Continued Process Verification (FDA/WHO) [5, 44] Ongoing Process Verification (EU/PIC/S) [4, 9]	Ongoing assurance that the process remains in a state of control	Continued Cleaning Process Verification

Table 4.3 provides a cross-reference between cleaning lifecycle phases and this ISPE Guide chapters relevant to the topic.

Table 4.3: Cleaning Validation Lifecycle Topics and Chapter Cross-References

Lifecycle Phase	Topic	Chapter References
Design	1. Define requirements and risk-based approaches	2, 3, 4.1.1, 5.6, 6.1, 6.2, 6.3, 6.4, 6.5, 7.4.1, 10.4, 11.1
	2. Complete residue characterization	4.1, 4.1.1, 5.1, 5.6
	3. Select cleaning agents	4.1.1, 5.2.7, 5.5, 5.6.2, 5.7
	4. Select cleaning process parameters	4.1.1, 5.5, 6.5.3, 10, 17, 18
	5. Select cleaning methods	4.1.1, 5.1, 5.4, 5.6.2, 5.7
	6. Review equipment design	5.2, 5.3, 10
	7. Determine residue limits	6
	8. Select analytical methods	5.6.2, 8
	9. Select microbial methods	8.2
	10. Define sampling methods and complete recovery studies	6.5, 7
	11. Determine acceptance criteria	6
	12. Complete small-scale studies	5.6.2, 5.8.1.1
Qualification	13. Determine readiness of equipment, methods, utilities	4.1.2, 4.4, 8
	14. Define validation strategy, number of runs	3.2, 4.1, 4.2, 4.3, 4.4, 5, 5.6, 6.4
	15. Define sampling plan	4.1.2, 4.4, 7
	16. Define visual inspection criterion	6.3
	17. Complete SOPs	4.2, 4.4
	18. Complete cleaning validation protocol	4.4
	19. Complete personnel training	4.1.2, 5.4, 6.3
	20. Execute validation runs	4.4
	21. Complete validation report	4.4
Continued Verification	22. Execute cleaning monitoring	4.1.3, 6.3.1, 6.4
	23. Execute periodic reviews	4.5
	24. Execute product changeover procedures	4.1.3
	25. Evaluate change control	4.6, 11

4.1.1 Cleaning Process Design

During the process design stage, the cleaning process variables are set, the criticality of parameters are assessed, and CIP/SIP cycle development (as applicable) and scale-up activities are completed. Small-scale, pilot, or laboratory studies are conducted to support the development effort in defining the cleaning process. The cleaning process design effort can be documented in a validation strategy document or plan. This overall strategy entails understanding the regulatory and operational requirements for cleaning, and any other equipment-related considerations (refer to Chapter 5).

Important Stage 1 elements are:

- **Residue Characterization:** An evaluation is performed to determine the characteristics of the soils to be cleaned (product, excipients, location, cleaning agents, dyes, foreign material, etc.). Aspects such as solubility, concentration, cleanability, and conditions for degradation or deactivation of residues are considered in the assessment.

- **Select Cleaning Agents:** Selecting cleaning agents is one of the first steps in developing a cleaning process. There are many options (e.g., water, solvents, chemicals, formulated cleaners). Knowing what needs to be cleaned (product, residues, cell growth media, proteins, dyes, etc.) and the type of surface to be cleaned will help in selecting the appropriate cleaning agent for the application. The cleaning agent should be compatible with the MOC of the surface to be cleaned, and have the appropriate capacity to dissolve, loosen, or impact the product/soil characteristics to be removed. Some cleaning agents are considered a type of residue in the cleaning program, and need to be removed below the established cleaning and SL. The selection of cleaning agents should be justified by laboratory studies that take into consideration the type and condition of the soil to be cleaned, the cleaning agent parameters (e.g., time, action, concentration, temperature) and the cleaning method to be used. Another consideration for selecting a cleaning agent is the level of degradation of APIs by the detergent. The extent of degradation may impact toxicity assessments and the analytical methods needed to support the cleaning process.

  The environmental impact of the disposal of cleaning agents into process streams or treatment facilities is an important consideration. Cleaning agent suppliers play an important role in ensuring consistent material quality. Suppliers may use different components in their manufacturing process that may impact the type of residues a company needs to clean.

- **Select Cleaning Process Variables and Justification of Criticality:** The identification of cleaning process parameters and justification of which ones are critical for process effectiveness are important elements to define during this stage. Typical parameters include:

  - Concentration of cleaning agents
  - Sequence of cleaning agents if more than one is used
  - Volume
  - Temperature
  - Time
  - Cleaning agent force (velocity for physically removing residues)
  - Number of cleaning cycles required
  - Need for initial rinse cycles

- Recirculation rinses
- Duration of blowdowns or drying cycle

Process parameters that can directly impact the effectiveness of a cleaning method should be considered critical. The objective is to define the cleaning process by defining the normal operating range for critical parameters, understanding the interactions between parameters, and determining any point of failure for the intended operating ranges. DOE methodologies can be used to determine the optimum ranges of cleaning process parameters in automated systems to prevent over-extending the time required to clean equipment. DOE can also accelerate the development of CIP cycle parameters. A short and effective cleaning process will maximize production capacity and overall productivity. A safety factor or contingency should be added to optimal productivity cycles to ensure the safety HBEL values are always met.

- **Select Cleaning Methods:** During Stage 1, the cleaning methods are selected, such as:
  - Manual cleaning
  - Clean out of Place (COP)
  - CIP
  - Soaking
  - Ultrasonic treatments
  - Pressurized wash
  - Flooding
  - Pre-cleaning treatments
  - Immersion in cleaning agents

  Refer to Chapter 5 for more details on cleaning methods.

- **Review Equipment Design and Inspection Procedures:** The knowledge developed in laboratory and pilot plants needs to be assessed against the equipment design and inspection procedures to ensure that the recommendations are properly applied and implemented. Equipment design and inspection procedure considerations include:
  - Coverage tests (e.g., riboflavin tests)
  - Results of laboratory test coupons
  - Condition of equipment surfaces (e.g., free of scratches, free of rouge)
  - Piping drainability
  - Dents
  - Surface finish
  - Level of light to conduct visual inspections

- Internal vessel configurations
- MOC

Dead legs need to be identified and opportunities for hold up of residues that require equipment to be dismantled for cleaning (as is common in solid dose manufacture) need to be identified and then either designed out if possible or dismantling built into cleaning processes. Flow rates through piping should be sufficient to prevent accumulation of residues and ensure full coverage of solutions. The MOC should be based on the process and the ability to clean. Stainless Steel (SS) vessels are common in the pharmaceutical industry, and are susceptible to corrosion under certain conditions. If groupings or bracketing of equipment are planned to simplify the validation effort, an equipment grouping comparison should be completed as part of the design review. Equipment groupings or bracketing can be justified for the same type of equipment (e.g., size, configuration, MOC, complexity) and the same cleaning procedure.

- **Determine Residual Limits and Overall Acceptance Criteria:** Acceptance criteria for residual limits should be set before assessing other parameters. Refer to Chapter 6 for a detailed explanation on the determination of limits and acceptance criteria. The overall cleaning acceptance criteria should include not exceeding the HBEL, cleaning limit (including action levels to ensure cleaning process control), and microbial control parameters, as well as achieving a successful visual inspection of selected cleaned surfaces. Refer to Section 6.3 for a detailed explanation of visual inspection.

- **Select Analytical Methods:** Selection of analytical methods is made taking into consideration method selectivity (for product specific methods), LOD or LOQ, and the method validation status. All methods should be validated before use. Specific methods are preferred; however, if it is not feasible to test for specific product residues, other representative parameters may be selected, for example, TOC. Refer to Chapter 8 for more details on analytical methods.

- **Select Microbial Methods:** The selection of appropriate microbial methods and the determination of initial alert level and action limits, sampling methods justification and selection, and necessary recovery studies are defined in this stage. Refer to Chapter 8 for additional details on microbial methods.

- **Define Sampling Methods and Recovery Studies:** Sampling methods are selected during the cleaning process development. Refer to Chapter 7 for additional information regarding sampling methods, and Sections 6.5, 7.1, and 7.2 for information on recovery studies.

4.1.2 Cleaning Process Performance Qualification

Stage 2 of cleaning validation can be divided in two subsections. Stage 2.1 is concerned with equipment readiness, including utilities. Stage 2.2 addresses the qualification and performance of the cleaning process.

The important aspects of Stage 2 are:

- **Determine Readiness of Equipment, Analytical Methods, and Utilities:** Before validating processes, the technical systems, equipment, utilities, and other supporting systems need to be qualified. Equipment qualification precedes validation activities and consists of challenges and tests to verify that the equipment was constructed per design, installed properly, and operates as intended within its required functionality. Qualification protocols are executed to document equipment readiness. Important for cleaning processes is the verification of proper operation of spray devices and their coverage. Visualization agents (e.g., riboflavin tests) are effective in verifying spray nozzle coverage. The qualification of automation and computerized systems is also verified (programmable logic controllers, CIP automated systems, etc.). These qualification activities need to be completed prior to starting cleaning process performance qualification runs.

  Utilities and corresponding distribution systems critical to the cleaning process need to be qualified. These include water (such as those defined in the USP [46]), clean steam, and gases (e.g., nitrogen, air).

Analytical methods readiness includes identification of those analytical methods that need to be validated and the methods to be transferred to the appropriate testing laboratory. Sampling methods via rinse or swabs should also be validated. Refer to Chapter 8 for more details.

- **Define Validation Strategy, including Cleaning Validation Protocol and Number of Runs:** Approved protocols are required to execute a cleaning PPQ. They consist of executing the cleaning process on soiled equipment to demonstrate the effectiveness and reproducibility of the cleaning process. The protocol should define and justify the number of cleaning validation runs, CHT (the amount of time cleaned equipment can remain in a cleaned state), Dirty Hold Time (DHT: the amount of time soiled equipment can remain dirty before cleaning), and drying time as part of the cleaning process. Refer to Chapter 5 for additional details.

  A cleaning PPQ needs multiple successful execution runs of the cleaning process to demonstrate process consistency. The number of PPQ runs to validate a cleaning method should be risk based and requires a documented rationale established on an understanding of the cleaning process, data obtained from the design and development of the cleaning process, and data from similar cleaning methods.

- **Define Sampling Plan:** A sampling plan describing sampling locations, number of samples, and the types of samples is prepared and integrated into the cleaning PPQ protocol for execution. Refer to Chapter 7 for more information on sampling.

- **Complete SOPs and Determine System Parameters:** SOPs describe the steps and specific instructions required to execute a cleaning process, and should be approved prior to starting PPQ runs. Automated systems have system configurations with appropriate parameters defining the sequence of steps to be executed for cleaning. These parameters define recipes for cleaning and should be clearly documented.

- **Complete Personnel Training and Qualification:** Personnel (i.e., operators, laboratory technicians, samplers, and inspectors) have the necessary education and experience, and are trained on SOPs related to the cleaning process and on the cleaning validation protocol content and objectives. If necessary, the training program includes operational details to ensure equipment is dismantled and assembled correctly. Personnel also receive on-the-job training to be able to execute procedures, document observations properly (e.g., visual inspections), and detect unexpected events. Effectiveness of on-the-job training is important to document the ability to execute the cleaning procedure as written. Training always precedes qualification.

- **Execute Cleaning PPQ:** Approved cleaning protocols are executed, and data (e.g., parameters, observations, CIP cycle start/stops) is documented for assessment against critical cleaning method process, critical parameters, and acceptance criteria. Refer to Section 4.4 for additional details. Cleaning validation is not required for clinical batches; instead, cleaning verification testing is performed based on the knowledge and criteria developed during Stage 1.

- **Complete Final Cleaning Validation Report:** The final validation report consists of several sections including:

  - Report approval
  - Executed protocols
  - List of products and equipment included in the cleaning validation study
  - Validated parameters and ranges
  - References to the cleaning procedures used
  - Discussion of failures and investigations that occurred during validation (visual and analytical results)
  - Plans for CPV and periodic reviews

- Conclusions

An important consideration should be an assessment of the final level of risk from the hazards and controls as demonstrated during the qualification runs. Future changes to equipment or product introductions will require ongoing analytical assessment, especially for low HBEL products. A justification should be documented in the validation report if the assessment of the final level of risks is deemed unnecessary. Prior to issuing a final report, interim reports may be issued. The interim report is similar to a final report (including QA approval) except that it only relates to some of the qualification runs from the cleaning plan.

4.1.3 Cleaning Process Continued Verification

During this stage, the cleaning process is monitored to ensure it is operating in a state of control. The main aspects of Stage 3 are:

- **Ongoing Cleaning Monitoring:** Through process monitoring, unplanned events representing a departure from the validated process can be detected. Data from process monitoring should be trended to detect shifts in cleaning method performance. The monitoring program should be risk based and mindful of the hazards and level of confidence established through the cleaning validation. The monitoring program can be a subset of tests utilized during the cleaning PPQ, and should include a sampling plan and should list all of the analytical methods to use. Refer to Section 6.4 for additional information regarding process control.

- **Periodic Review:** A comprehensive evaluation of the cleaning process state is performed as part of the validation lifecycle. The intention of the review is to demonstrate that the cleaning process remains in a validated state of control. The outcome of a periodic review may include recommendations for process improvement or for revalidation if the cleaning process is found out of control. Refer to Section 4.5 for more details.

- **Product Changeover Procedures (PCO):** PCO procedures should be in place. Risk-based methodology, along with historical performance, should be used to determine when to initiate PCOs.

- **Additional Controls:** Validated cleaning processes are subject to change control. Refer to Chapter 11 for more details. A preventive maintenance program is in place to maintain the equipment in an operational state. In addition, unexpected events representing failures to the cleaning process are documented as deviations. For example, an unsuccessful manual cleaning that does not meet the acceptance criteria must be documented prior to recleaning following an approved procedure. This procedure should point to an investigation requirement that tracks and trends all failures similar in nature to further identify root cause so that appropriate corrective and preventive measures are applied rather than just repeating the cleaning until all acceptance criteria are met [4]. These deviations are investigated to assess the probable causes and corrections to bring the cleaning process back to a state of control. Preventive maintenance and calibration programs keep equipment and instruments operating correctly and in a calibrated state.

4.2 Documentation

The effective management of documentation is essential to comply with GMPs. There are business, regulatory, and scientific reasons to have good control of documents. Good documentation practices ensure traceability of the development, qualification, and maintenance of cleaning processes and enables the company to capture and manage knowledge.

FDA [5] defines validation as:

> *"The collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality products."*

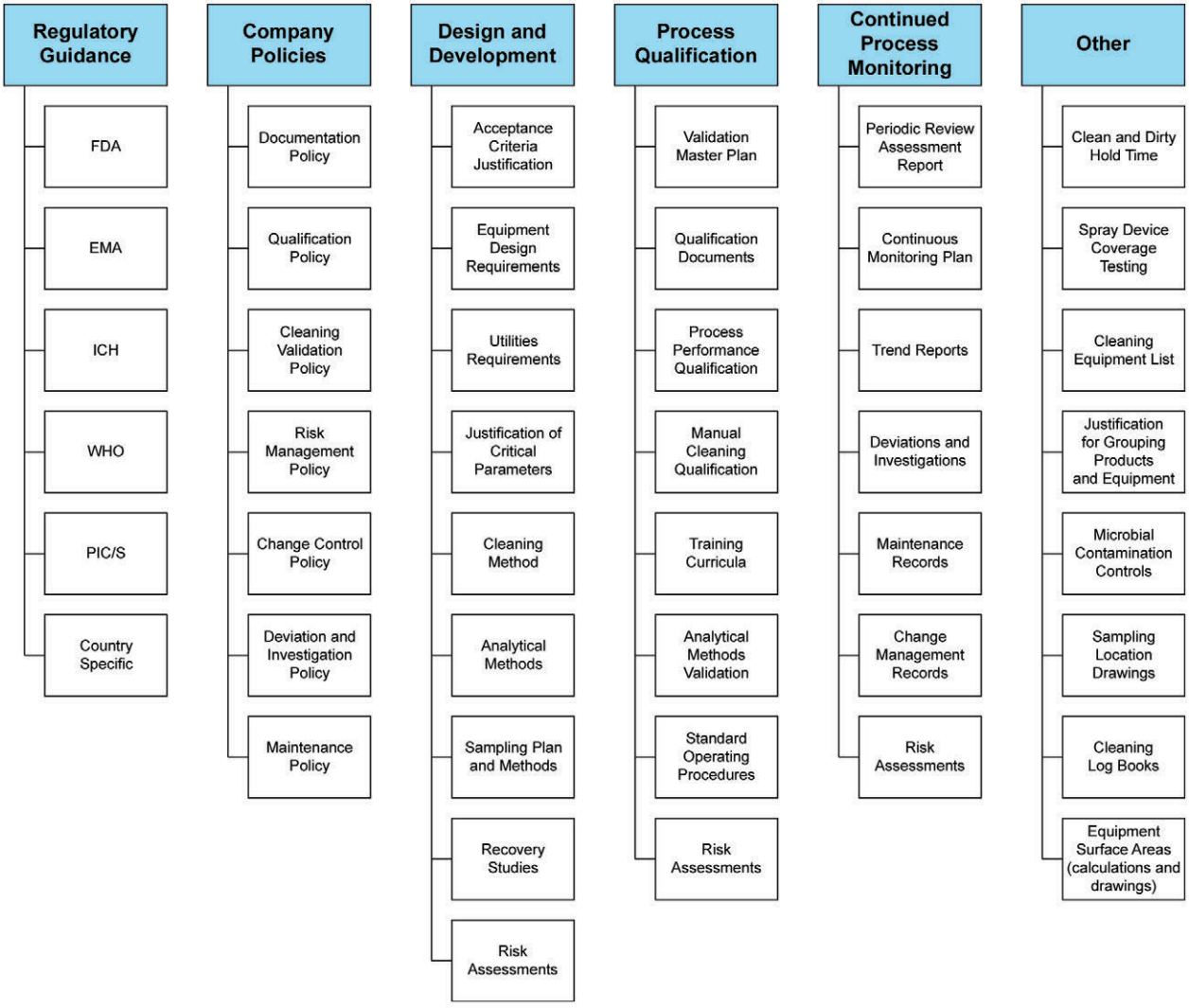
PIC/S [47] and EudraLex Annex 15 [4] define cleaning validation as:

> *"Documented evidence that an approved cleaning procedure will reproducibly remove the previous product or cleaning agents used in the equipment below the scientifically set maximum allowable carryover level."*

In all the definitions cited above, it is essential to validation to document evidence and collect data accurately, preserve its integrity, and evaluate it to demonstrate that a cleaning process works as intended.

Figure 4.2 describes typical documents associated with developing, qualifying, and maintaining cleaning processes and controls. In general, there is a hierarchy for documentation that starts with company policy and regulations, followed by high-level validation plans, validation protocols, SOPs, laboratory studies, and records.

Figure 4.2: Typical Documentation Associated with Cleaning Validation



4.3 The Validation Master Plan

The VMP states the overall philosophy, intentions, and approach used by the facility for planning, designing, organizing, executing, and reporting validation and qualification activities. The plan is updated periodically to ensure it includes the current state of validation.

The general format of a typical VMP addresses the following elements:

- Cover – Identifies the system or facility described in the plan
- Approval – Identifies the document reviewers and approvers
- Introduction
- Description of validation philosophy and approaches (use of family approaches, methods for defining worst-case conditions, lifecycle of validation, etc.)
- Organizational structure
- Roles and responsibilities
- Description of facility
- Description of manufacturing operations
- Description of key validation procedures
- Guidance on setting and justifying acceptance criteria
- List of qualifications (equipment, utilities, components, etc.)
- List of validations (cleaning, process, analytical methods), maintenance procedures
- References – List of references used to develop the VMP, relevant operating procedures, and policies
- Attachments – Other references such as facility layouts
- History – Documentation of revision history of the VMP

A Cleaning Validation Plan (CVP) can be created as a separate document or included in the site VMP. For cleaning validation, the following additional topics are addressed:

- Surfaces to be cleaned (MOC)
- Product or residue attributes (material to be cleaned) including product formulation information and chemical/physical attributes
- Approaches to product grouping (similar products or residues can be grouped to simplify validation approach)
- Approaches for equipment grouping (equipment may be grouped by similar designs, similar functions, or worst case for cleaning)
- Type of cleaning process (manual, automated, or hybrid)

- Residue limits, visual inspections, LOD, LOQ, and worst-case justifications
- Cleaning parameters
- Sampling types and methodologies (swabs, rinse, sampling location justification)
- Frequency of cleaning

4.4 Creation and Execution of Validation Protocols

The correct execution of protocols is crucial for obtaining the data needed to validate processes. The cleaning validation protocols are written after the cleaning process has been developed. Cleaning PPQ execution cannot begin before approving the validation protocol.

The content of the protocol is product and method-specific to ensure that evidence is collected for validation. Protocol content includes:

- Cleaning procedure to be validated
- Products covered by the protocol; any grouping if used
- Description of equipment and equipment surface area
- Residue materials to be removed
- Cleaning and sanitizing materials used
- Number of runs with rationale, and campaign lengths, if applicable (i.e., validation strategy)
- Cleaning parameters and CPPs to be evaluated
- Analytical methods
- Microbiological methods, if applicable
- Sampling plans, including rationale for the selection of specific sites identified for sampling
- Sampling methods
- Acceptance criteria, reference to calculations, if applicable
- Maximum time interval between use and cleaning, or between cleaning and use, if applicable (i.e., DHT, CHT)

Trial or practice runs are recommended (e.g., for new or complex systems) prior to protocol execution to verify that all the elements of the cleaning process have been properly implemented. Trial runs intended to represent commercial manufacture should include all the controls of the cleaning validation program. Protocol deviations can be minimized during execution by ensuring operational readiness. (Refer to Table 4.4 for a summary of points to consider.)

Table 4.4: Points to Consider when Executing Validation Protocols

For Protocol Readiness	For Operational Readiness	Protocol Execution
• Confirm that written procedures (SOPs) detailing the cleaning process are approved • Verify that recovery methods are ready, analytical equipment is qualified, sample storage conditions are defined, and analytical procedures are approved • Verify that protocol is complete (cleaning method, parameters, sampling plan, number of runs, acceptance criteria, roles and responsibilities are defined, etc.) • Confirm that testing scripts are clearly defined and documented • Clarify when cleaning protocol execution will stop collecting samples or data (end point) • Ensure that protocol is approved prior to execution	• Conduct test or dry runs (correct errors in protocol, confirm that all key steps are included) • Ensure that equipment is qualified and available to conduct cleaning validation • Confirm that utilities and their distribution systems are qualified and available for cleaning operations • Verify that relevant instruments are calibrated and ready for use • Verify that materials, chemicals, utensils, etc. are available for use • Confirm that system for discrepancies and issues management is in place • Complete qualification and training for all persons participating in protocol execution • Ensure Quality Control (QC) laboratories are ready to receive samples for analytical testing • Secure personnel support (e.g., weekends, shifts covering 24 h/day) for the duration of the protocol execution • Qualification reports are satisfactory and approved before Performance Qualification (PQ) execution	• Follow good documentation practices (unambiguous date format, write legibly, no empty or blank spaces) • Use indelible ink when documenting on paper • Collect data accurately, contemporaneously • Document unexpected events or observations immediately • Ensure traceability and chain of custody for validation samples • Ensure sensitive samples (e.g., non-stable, with expiry dates) arrive on time to the testing laboratory • Securely save or file executed validation protocols and attachments after protocol execution

Usually multiple successful cleaning process runs are required to generate the data required for validation. The number of runs required to demonstrate reproducibility and consistency of the cleaning process should be justified based on the process variability and risk. When one or more of the multiple runs fail to meet acceptance criteria, an impact assessment to the cleaning PPQ is necessary.

When cleaning process failures occur, an investigation is initiated to determine the root cause. If the root cause for the failure is related (intrinsic) to the cleaning process, completion of the validation PPQ may be impacted. In order to demonstrate process consistency, consecutive batches are manufactured as evidence. An intrinsic process deviation resulting in a failed cleaning acceptance criterion usually requires extension to the validation by an amount dependent on the nature of the failure and the need to prove consistency of the cleaning process. For example, for automated cleaning processes, the consecutiveness of successful runs during validation is expected. However, for manual processes, a validation approach using consecutive runs may give a false assurance of consistency, and would require a more robust approach to demonstrate validation that takes into consideration the variability between operators.

If the deviation and root cause are extrinsic to the cleaning process, then the consecutiveness of cleaning PPQ runs is normally not interrupted. These decisions are made on a case-by-case basis given the large variety of cleaning methods and equipment/product grouping strategies. However, the decisions should follow certain principles illustrated by these questions:

- Is the root cause for the failed cleaning event intrinsic or extrinsic to the cleaning process?
- Were product quality attributes negatively impacted by the failure?
- Can corrections be implemented to avoid recurrence?

Deviations to cleaning methods or failure to achieve the cleaning acceptance criteria requires investigation according to established deviation procedures. Root cause for the failures and corrective actions are then identified and implemented prior to starting additional cleaning PPQ runs.

All documents created for protocol execution are managed under GMP using the documentation management system of record.

4.5 Periodic Reviews

A Periodic Review (PR) of the cleaning validation process is performed as part of the cleaning lifecycle. The comprehensive review includes multiple data sources relevant to cleaning method control. The frequency of the PR is determined by a risk assessment taking into consideration how frequently the cleaning process is executed, the failure rate for cleaning, and the impact a cleaning failure can have on the company or product supply. The review is conducted formally by SMEs and overseen by the quality unit. All data collected, assessments made, and recommendations are captured in a PR report.

A typical PR report consists of the following:

- Cleaning Process Description
- Scope of the review
  - History of cleaning process changes
  - Evaluation of cumulative impact of changes
  - Summary of critical parameters monitoring
  - Summary of alarms of critical parameters or events
  - Results of routine monitoring
  - Review of non-routine maintenance and events
  - Review of deviations and corrective actions, including visual inspection failures and trends
  - Review of frequencies of recleaning cycles and justification
  - Review of changes to analytical methods
  - Review of equipment inspections and maintenance (scratches, rouging, damages, leaks, etc.)

- Review of data trends
- Review of risk assessments supporting cleaning validation
- Review of Cleaning Process Risk Assessment
- Review of SOP changes and training conducted during this period
- Evaluation of changes in regulations impacting the cleaning process

• Analysis of the data collected (per scope) and criteria to determine if process is in control

• Conclusion of the review and recommendations

A robust monitoring program should provide sufficient assurance that a cleaning process is operating in a continuous validated state. However, the monitoring seldom challenges key validation requirements such as sample location, deviations, or the impact of a new product introduction to a facility. For this reason, a comprehensive PR process is recommended to ensure all key elements of the cleaning process are still adequate and in control.

4.6 Cleaning Revalidation

The decision to revalidate a cleaning process usually is taken in the context of changes introduced to the system. (Refer to Chapter 11 for details on change control.) Significant changes impacting cleaning parameters, analytical methods, new technology, or the ability to execute the process may require revalidation. However, not all triggers for revalidation come from individual changes managed via the change control system. The PR process can assess holistically all the elements used to control the cleaning process during a long period of time (usually years) and determine if it is still operating in a validated state or if there are shifts from its expected performance. The extent of the revalidation scope can be limited to certain aspects of the cleaning process (e.g., verification of cleaning hold times), based on the PR assessment.

5 Cleaning Methodologies

5.1 Selection of Cleaning Process

The cleaning process must reduce residues to levels that ensure patient safety and result in visibly clean equipment. An effective cleaning process can reduce equipment downtime, increase the lifetime of the equipment, and minimize the risk of cross-contamination in multiproduct facilities. The objective of development should be a cleaning process that is rugged enough to clean the worst-case soil to levels well below the cleaning limit. The cleaning process should also be rugged enough so that when new products and chemicals, and their resulting residues are introduced into the facility, the existing cleaning process is effective, thus eliminating the need to develop additional cleaning processes and the resulting cleaning validations.

Many factors should be considered during the development of a cleaning process, most importantly, what cleaning method to choose. Cleaning methods are broadly divided into three groups:

- CIP applications – Section 5.2
- COP applications – Section 5.3
- Manual cleaning – Section 5.4

Manual cleaning may be selected for cleaning small parts, parts with small lumens, and delicate parts such as pressure gauges. It involves either wiping the equipment down, cleaning at the sink with a brush, using soaking tanks, or using ultrasonic baths. Manual cleaning is relatively simple; however, it may be inconsistent due to operator-to-operator variability and may be labor intensive if a large number of parts are cleaned in this manner. Small parts such as gaskets, small stainless parts, filter housings, glassware, and carboys are well suited for cleaning using automated parts washers. The advantage of parts washers over manual cleaning is having reliable performance, reduced labor, and no operator exposure to cleaning agents. Large equipment may be cleaned in place or out of place using automated CIP systems or manual methods such as pressure spray, foaming, brushes, or wiping. Cleaning method choice may be restricted due to constraints in building utilities such as the availability of hot water, drains in the area, and mechanical air.

Four key factors needed for reproducible, validatable cleaning are frequently referred to by the acronym TACT (Time, Action, Chemical, and Temperature).

Time – A reproducible cleaning process requires the same duration each time it occurs with regard to phases such as rinses, chemical solution wash, and draining. The criticality of processing times should be evaluated during process development and validated against the target for cleaning validation.

Action – A reproducible cleaning process requires the same surface action each time it occurs. The flow rate/pressure can be quantified within a reasonable range, such that the greatest and least flow rate/pressure are evaluated during development studies and validated at the designed flow rate/pressure. During manual cleaning, action is the type of force on the surface ranging from soaking to agitated immersion or the mechanical action of brushing or wiping.

Chemical – Cleaning agents must be selected for efficacy. The reproducible rate kinetics of cleaning chemicals are affected by concentration. Chemical concentration can be qualified by solution concentration formulation and verified by solution conductivity and/or pH when those analytical characteristics are active to the particular detergent chemical. The chemical concentration can be quantified within a reasonable range, such that the greatest and least concentration conditions are evaluated during coupon development studies and validated at the designed detergent concentration.

Temperature – The reproducible rate kinetics of cleaning agents can be affected by temperature. A reproducible cleaning process requires the same temperature each time it occurs. The temperature can be qualified within a reasonable range during coupon studies, such that the highest and lowest temperature conditions are evaluated and validated at the designated operating temperature.

A rugged cleaning process is directly impacted by the amount of cleaning action on the surface of the equipment [48]. CIP systems rely on a combination of spray impingement, cascading flow, and agitated immersion for cleaning whereas manual cleaning generally exerts more scrubbing action on the surface. Selection of the method should take into consideration the size and quantity of the equipment, design of the equipment, and the level of automation available at the facility.

Development of a Cleaning Process

Development of a cleaning process should include a review and risk assessment of residue characteristics. Review the process development or historical cleaning data, if information is available, to determine the physicochemical characteristics of the residue. Information may also be leveraged from products having similar characteristics or the same products having different cleaning methods.

Consideration should be given to the API; however, in some cases, the API will not be the hardest-to-clean active or the most prolific component in the formulation. For that reason, a thorough review should include any raw materials, excipients, impurities, degradants, or by-products that may contribute to a harder to clean or more toxic residue. The API should be assessed by reviewing pharmacological (represented by the ADE/PDE/HBEL as available) and solubility data, along with its strength or percentage in the formulation.

Review and understand the manufacturing process including processing temperatures, process hold times, and environmental temperatures to understand how these may impact the residue characteristics. Soil condition on the equipment and MOC function together and should be considered when developing the cleaning process. The amount of soil on the equipment should be consistent among batches to ensure consistently adequate cleaning. Depending on the equipment, this can be accomplished using a clean-out step at the end of manufacturing. Scraping and/or vacuuming the equipment can remove the majority of the soil. This serves two purposes: providing a consistent soil level for cleaning and reducing the amount of soil flushed down the drain into the wastewater treatment facility.

Develop an equipment matrix to understand grouping, design, and product contact MOC.

Determine available cleaning process options since there may be limitations with utilities, waste requirements, and automation. Review and understand any restrictions that may influence the cleaning method.

- pH restrictions due to MOC or waste stream limitations
- Temperature restrictions due to MOC or waste stream limitations
- Decontamination step that may change the characteristic of the residue
- DHT constraints

Additional considerations for cleaning process development include: contribution to microbial growth, equipment availability, and manufacturing schedule needs.

5.2 Clean in Place (CIP)

5.2.1 Principles of CIP

The underlying principle of CIP is to remove undesirable actives and chemicals from product contact surfaces by mobilizing the soils away from the surface and into a suspension or solution. The mobilization occurs by flowing or spraying rinse and/or detergent solutions over the surfaces to be cleaned, achieving a combination of impingement and cascading surface action and solubility. CIP generally refers to automated systems consisting of spray devices and cleaning agent delivery controlled via HMI/SCADA.

5.2.2 CIP Defined

CIP is the removal of undesirable actives and chemicals from product contact surfaces in their process position, without the need for disassembly to obtain access to the surface being cleaned. Those components that cannot be cleaned in their process position by CIP are removed from the equipment for COP according to procedure. In most cases CIP uses aqueous-type rinse and detergent solutions. In some API processes, volatile solvents are required as a CIP solvent.

5.2.3 CIP-Cleanable Processes

Process equipment needs to meet specific criteria for design, fabrication, MOC, installation, inspection, and maintenance to be suitable for CIP cleaning. These criteria are detailed in Chapter 10.

Processing equipment and piping systems that are cleaned in place show less wear and tear (and damage) than comparable items cleaned manually. With automated CIP, the labor required for cleaning and maintenance is reduced and the processing system productivity is increased through a reduction of downtime. At the same time, reproducibility increases as automation replaces manual cleaning procedures.

While CIP-cleanable process equipment is important, substantial experience has shown that successful CIP implementation involves far more than the selection and application of CIP-cleanable components such as pumps, tanks, instruments, valves, etc. The design of a cleanable process requires the consideration of unit operation processing, equipment design conducive to CIP cleaning, process equipment layout, and the interconnecting piping design for the process to provide for proper cleaning via the configuration into CIP circuits. Specialized CIP systems may be required.

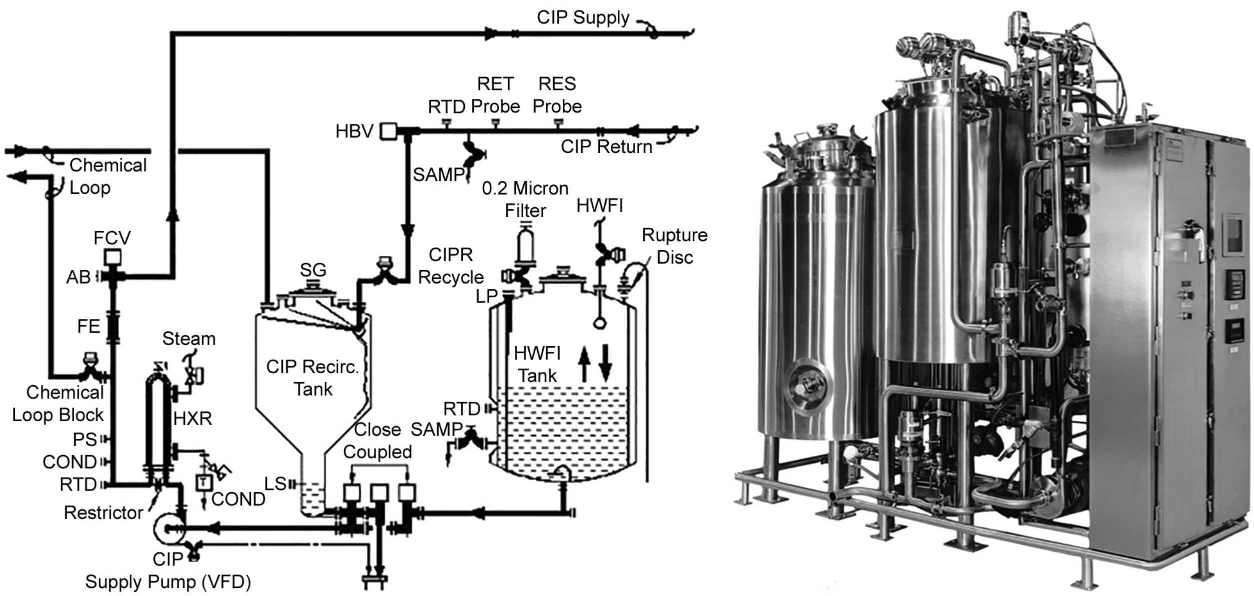
CIP is applied to various pharmaceutical processes both for liquid (biotechnology, parenteral solutions, IV solutions, blood fractionation) manufacturing equipment and solid (Drug Substance (DS), DP) manufacturing equipment. Liquid CIP equipment includes tanks, filters, and centrifuges; whereas solid CIP equipment comprises, for example, fluid bed driers, and apparatus for crystallizing, filtering, drying, milling, blending, and bulk container filling.

5.2.4 CIP Systems

The CIP system is a packaged system of properly integrated components typically including tank(s), pump(s), heat exchanger(s), chemical feed equipment, valves, instruments, and system controls. See Figure 5.1. This system is designed to provide automatically controlled spray cleaning operations of storage tanks and processing vessels and pumped recirculation washing of product transfer piping systems. The integrated system makes it possible to achieve complete and uniform control of the TACT key factors.

Practical field experience reveals that it is most commonly easier to pump water into process tanks than to get it back to the CIP system through CIP return. For a two-pump system, rinse and detergent wash solutions must be continuously removed from a vessel being spray cleaned at a rate equal to the solution supply to ensure the return pump does not cavitate. Inconsistent CIP return conditions create commissioning and validation challenges as TACT factors such as surface cleaning action, chemical solution concentration, and cycle time become unreproducible. Based on process design, facility layout, project budget, and other considerations, the engineer decides on the optimum configuration for CIP return conditions.

Figure 5.1: Example of a 2-Tank CIP System Showing Major Components
Used with permission Electrol Specialties Company (ESC), www.esc4cip.com.



Solution return may be accomplished by:

- Gravity
- Return pump
- Eductor (vacuum created by Venturi flow effect) motive force
- Some combination of the above

Gravity Return Flow – Gravity CIP return is applicable when the tank being cleaned is at one or more levels above the recirculating unit. Tank outlets and return piping systems need to be sized large enough to permit return by gravity alone. When properly engineered, gravity drainage is more effective and dependable than other methods for removing the final traces of liquid from a CIP circuit.

Pumped Return Flow – Low-speed (1500 rpm) centrifugal return pumps, either with the head rotated by 45° from vertical or of a “self-priming” type, provide effective and reliable return flow <u>if</u> the return header pitches continuously from the tank being cleaned to the pump inlet with sufficient Net Positive Suction Head (NPSH). CIP return pump performance may be reduced by elevated water temperature.

Eductor-Assisted Return Flow – The combination of a motive pump and eductor incorporated at the CIP system establishes eductor-assisted CIP return flow systems. An eductor-assisted CIP system will pump both air and water and create vacuum CIP return flow motive force.

Combination – While eductor return flow can be used as a sole motivating force for short CIP return runs involving minimal static head, an eductor is most commonly used in conjunction with gravity return or a CIP return pump. The eductor continuously primes the return pump, which in turn is able to handle an air-water mixture. Eductor performance, relative to the vacuum capacity being generated, decreases at elevated water temperature, but usually retains sufficient vacuum to assist with overcoming hold up in the process tank being cleaned and to prime the CIP return pump.

Proper application and engineering with respect to the CIP system, return flow motive force, and piping installation make it possible to conduct CIP cleaning operations with a high degree of uniformity and reproducibility. See Figure 5.2.

Figure 5.2: Example of Tank and Line CIP Circuits with CIP Recirculating System on Left, Process Vessel at Center, and Process Piping on Right

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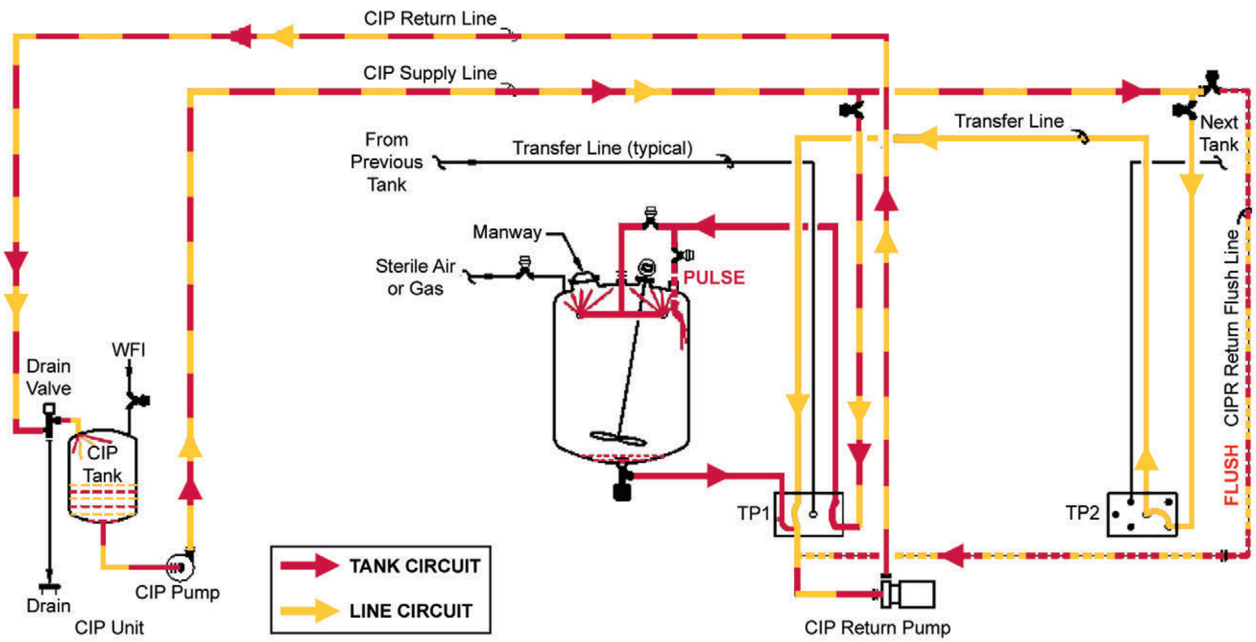


5.2.5 CIP Flow Rates in Piping

CIP solution flow in piping systems must have sufficient flow rate velocity to ensure that the line is fully flooded and experiencing a turbulent scrubbing action.

Turbulent flow is defined as a Reynolds number that exceeds 4,000. The Reynolds number gives a measure of the ratio of inertial forces to viscous forces. With a Reynolds number greater than 4,000, the flow of the CIP fluid can be anticipated to receive sufficient turbulent action [49].

However, the Reynolds number does not address factors such as branches, tees, elbows, and clearing air pockets from long horizontal piping runs. If the cleaning solution is not in direct turbulent contact with the surface being CIP cleaned, effective reproducible cleaning does not occur.

The generally accepted guideline found to be adequate for cleaning flow rates in a variety of processes is to achieve a velocity of 1.5 m/s [32].

The 1.5 m/s guideline is applicable to piping systems with branches where [32]:

$L/D \leq 2$

Where: L = Length of branch extension, such as to the face of a capped tee or to a valve weir
D = inside Diameter of the extension or the nominal dimension of a tee, or valve

Also, the leg extension must be in the horizontal plane since an upward extension traps air and a downward extension traps particulate. In cases where the L/D and/or leg orientation are otherwise than recommended, increasing velocity to on the order of 3 to 5 m/sec may allow sufficient cleaning solution in direct turbulent contact with the surface being CIP cleaned. However, in some piping geometries with excessive leg extensions the affected area is not suitable for CIP cleaning.

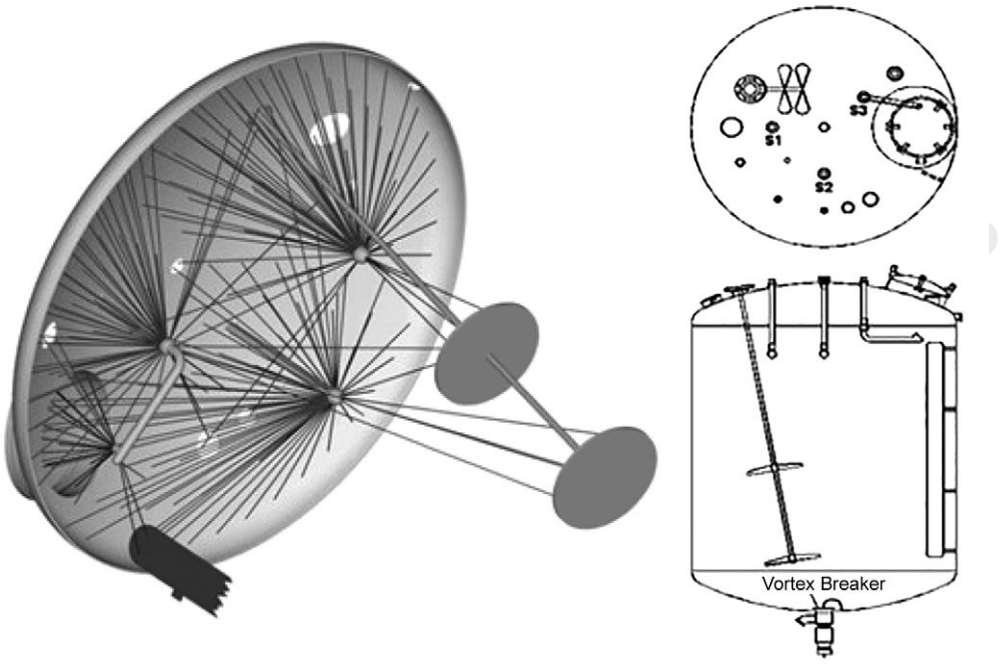
5.2.6 CIP Spray Devices

There are two primary types of CIP spray devices: static directionally drilled spray devices and rotating spray devices. Any spray device needs to be permanently etched and/or have pin locks so that when a fixed device is removed from a vessel for inspection, maintenance, etc., the spray device can only be placed back in the vessel in the correct orientation.

Static directionally drilled spray devices work on the principle of high volume, low pressure continuous action on all tank surfaces. This ensures all tank surfaces are receiving solution coverage all the time. An empirically determined guideline adopted by hygienic design accepted practices has been that vertical storage vessels require 9.5 to 11.3 LPM per linear foot of circumference [32]. Dished-head vertical vessels are cleaned with the majority of flow directed with spray devices toward the upper head and sidewall area at the knuckle radius. Gravity then provides for a continuous solution sheeting action over the side wall and bottom head.

Specific streams may be directed at internal components such as baffles, agitator impellers, tank sidewall nozzles, etc. In these cases, the extra spray stream flow rates are added to the 9.5 to 11.3 LPM to obtain the total spray flow requirement [32]. Static spray devices typically operate in the pressure range of 1×10^5 Pa – 2×10^5 Pa. The development of directionally drilled spray devices with coverage based on 3-D CAD modeling has opened the way to ensuring optimum coverage. See Figure 5.3. In this method, the process equipment 3-D CAD design model is overlaid with spray-device trajectory paths to locate both the spray devices and the directionally drilled individual holes to target difficult-to-clean areas and can provide for redundancy of coverage where necessary.

Figure 5.3: 3-D CAD Model on Left Showing Directionally Drilled Spray Device Streams for CIP of Process Vessel on Right, with Targeted Coverage of Nozzles, Manway, Agitator, and Baffles
Used with permission Electrol Specialties Company (ESC), www.esc4cip.com.



Static spray devices require scheduled maintenance for inspection to ensure that holes are not plugged. An example of a static spray device is shown in Figure 5.4.

Rotating spray devices work on the principle of low volume, high pressure action on the tank surfaces. Figure 5.5 contains examples of rotating spray devices. The spray nozzle is powered by the supply pressure of the CIP fluid to perform a geared rotation around the vertical and horizontal axes. Rotating spray devices typically operate in the pressure range of 3 to 8 Bar. With each cycle the nozzles lay out a narrow rotational pattern on the tank surface. The subsequent indexed cycles gradually make the pattern denser, until a full pattern is reached after multiple cycles, typically on the order of 20 to 50 revolutions over 10–20 min.

Rotating spray devices require sensor detection during CIP to ensure rotation speed, along with scheduled maintenance for replacement of seals and bearings, and inspection to ensure that holes are not plugged.

Regardless of whether static or rotating spray devices are utilized, it is necessary to ensure a match between the CIP spray-device flow rate and that required by the product piping. For example, it is important that the tank spray-device flow rate be commensurate with the flow rate needed not only for cleaning the process tank but also its tank outlet valve and downstream.

Figure 5.4: Example of Static Directionally Drilled Spray Device on Left with Index Pin Location to Ensure Reproducible Installation in Process Vessel on Right
Used with permission Electrol Specialties Company (ESC), www.esc4cip.com.

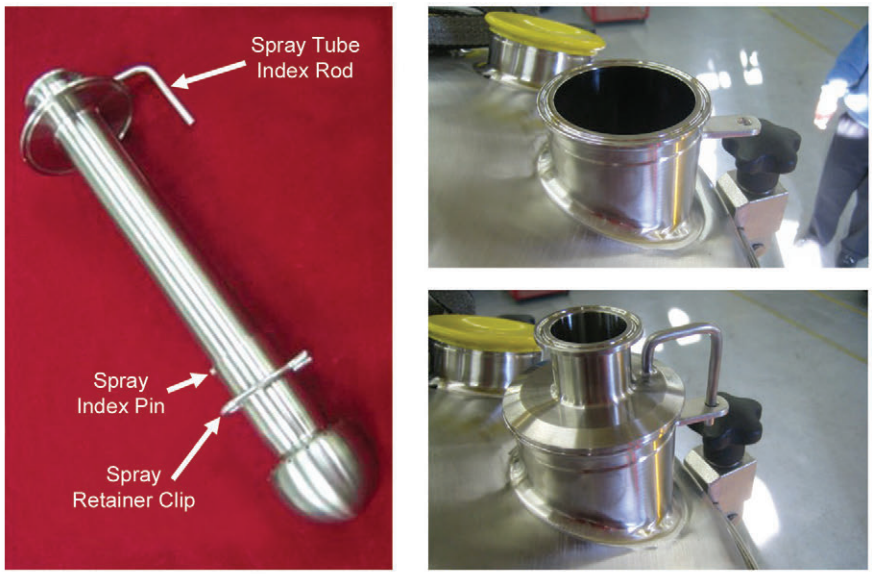


Figure 5.5: Examples of Rotating Spray Devices
Used with permission from Sani-Matic, Inc., https://sanimatic.com.

5.2.7 CIP Cycle Development

Cleaning cycles vary by industry; however, they are based on the same principles of relying on TACT. Various water-soluble solutions with different properties are used in sequence to obtain the desired cleaning effect. For example, alkaline solutions may be useful to break down proteins and fats, while acid solutions help to neutralize alkaline conditions and remove mineral deposits. The development of cleaning cycles takes into consideration the residue characterization information to decide the most effective combination of conditions and parameters. CIP units are usually optimized to avoid unnecessary waste of utilities and chemicals and to achieve cleaning in the shortest time possible. In-process instruments can measure solution pH, volume flow rate, turbidity levels, temperature, time, and pressure. These parameters can be used to define the start and end point of different cycles. The cycle development will select and justify the optimum sequence and combination of cleaning steps to achieve a consistent, robust, and validatable cleaning process.

The following CIP Cycle example is typical of aqueous-based cleaning used for biological type process residues that primarily consist of proteins, carbohydrates, lipids and water-soluble salts. CIP cycles for nonbiological, chemically synthesized process residues requiring solvent-based cleaning may vary greatly from the following example in that the cycle may consist partially or entirely of contacting and solubilizing the residue with one or more nonaqueous solvents.

Water used for CIP cycle Rinse and Wash Phases can be of the most available in the process area that is designated as suitable for use on product contact surfaces for cleaning procedures. Water used in the Final Rinse Phase should be of equivalent quality to that used in the subsequent process.

Example CIP Cycle

Pre-CIP Activities

Residual product material is removed from process equipment to the greatest extent possible, for example by draining in the case of liquids or dumping in the case of dry solids.

Process system components that are not suitable for CIP cleaning are removed for COP and taken for cleaning either manually or in a parts washer. If the location of the removed component leaves an opening where CIP solution would escape, the location is sealed or capped using in a CIP-cleanable method of closure.

Any manual connections that are necessary for completing the CIP supply and return flow path are completed using components such as hoses, piping spool pieces, and U-bend transfer panel jumpers. Manual valves are set to the necessary position required during CIP.

Start of CIP Cycle

The automated CIP Cycle should start following the end of production at a time not exceeding the validated DHT.

First Rinse or Initial Rinse Phase

During the initial rinse, easily detached soils are removed from the equipment surfaces. Frequently, the vast majority of active and chemicals are removed in this first step. For actives or chemicals containing proteins, the initial rinse water is typically at ambient temperature, to reduce the chance of denaturing the protein causing adhesion to the equipment surface. Conversely, for residues with a high lipid composition, a heated initial rinse may be advantageous to prevent congealing.

For spray-device contact circuits, a starting point for cycle development is on the order of three successive Rinse-Drain burst combinations of 30 s surface rinse contact time followed by complete vessel drain. Successive Rinse-Drain combinations help ensure that as non-soluble material rinses from the equipment, it is carried down the outlet to the CIP return. For process lines, a continuous initial rinse volume on the order of two times the total line volume is a typical starting point. Initial rinse duration is adjusted during cycle development based on achieving a balance of optimizing removal of easily detached residue against required cycle time duration and water consumption.

System Drain Phase

A Drain Phase allows for solution to drain completely from vessels and/or lines through the CIP return, improving the cleaning effectiveness of the subsequent step.

Alkali Detergent Wash Phase

The Alkali Detergent Wash is an alkaline aqueous-based solution alone or in combination with wetting agents and other additives. The Alkali Detergent Wash duration timer starts when the equipment surface is contacted by solution meeting the action (flow rate and pressure), chemical concentration, and temperature criteria of the TACT principle.

For spray-device contact circuits, a starting point for Alkali Detergent Wash cycle development is on the order of 10 min contact time. For process lines, a continuous detergent wash volume on the order of five times the total line volume is a typical starting point. The TACT parameters of the Detergent Wash are adjusted based on the recommendations of the cleaning compound manufacturer to achieve the requisite surface cleaning.

This step may be a single pass or recirculated where the cleaning agent is delivered to the equipment from the CIP system recirculation/wash tank and either is directed to drain or recirculated through the equipment and back to the CIP system recirculation/wash tank.

Gas Purge (Air Blowdown) and Drain Phase

The Gas Purge utilizes clean process air or nitrogen to clear the CIP supply lines and process piping of residual solution prior to supplying fresh solution in the next phase. A Drain Phase follows the Gas Purge Phase to allow the pressure to dissipate and for the solution to drain.

Intermediate Rinse Phase

An Intermediate Rinse is used to remove residual detergent wash solution from the equipment surfaces before proceeding with subsequent cycle phases.

For spray-device contact circuits, a starting point is on the order of two successive Rinse-Drain burst combinations of 30 s surface rinse contact time followed by complete vessel drain. For process lines, a continuous Intermediate Rinse volume is on the order of one time the total line volume.

Acid Detergent Wash Phase

The Acid Detergent Wash is an acidic aqueous-based solution alone or in combination with wetting agents and other additives. The Acid Detergent Wash helps to neutralize residual caustic, remove mineral salts and oxides, and establish a free-rinsing surface. The Acid Detergent Wash duration timer starts when the equipment surface is contacted by solution meeting the action (flow rate and pressure), chemical concentration, and temperature criteria of the TACT principle.

For spray-device contact circuits, a starting point for Acid Detergent Wash cycle development is on the order of 5 min contact time. For process lines, a continuous detergent wash volume on the order of two times the total line volume is a typical starting point. The TACT parameters of the Detergent Wash are adjusted based on the recommendations of the cleaning compound manufacturer to achieve the requisite surface cleaning.

Gas Purge (Air Blowdown) and Drain Phase

The Gas Purge utilizes clean process air or nitrogen to clear the CIP supply lines and process piping of residual solution prior to supplying fresh solution in the next phase. A Drain Phase follows the Gas Purge Phase to allow the pressure to dissipate and for solution draining.

Final Rinse Phase

During the Final Rinse Phase, process equipment surfaces are flushed free of process and chemical detergent residue along with lower quality water that may have been used in the preceding phases. For spray-device contact circuits, a starting point for cycle development is on the order of three successive Rinse-Drain burst combinations of 30 s surface rinse contact time followed by complete vessel drain. For process lines, a continuous Final Rinse volume on the order of two times the total line volume is a typical starting point. Final Rinse duration is adjusted to achieve the final validated state for cleaning, frequently based on an online rinse water solution resistivity and/or TOC criteria.

Final Gas Purge and Drain Phase

The Gas Purge utilizes clean process air or nitrogen to clear the CIP supply lines and process piping of residual solution prior to supplying fresh solution in the next phase. A Final Drain Phase follows the Gas Purge Phase to allow the pressure to dissipate and for a complete draining of the process equipment, process lines, CIP system, and CIP supply/return lines.

Post-CIP Activities

Process system components that have been removed for COP are reinstalled in their use location.

Any manual connections that are necessary for returning to subsequent process conditions are completed using components such as hoses, piping spool pieces, and U-bend transfer panel jumpers. Manual valves are set to the necessary position for subsequent process conditions.

Process sanitization or sterilization procedures follow if applicable.

Clean Hold Time (CHT)

The process CHT starts at the end of CIP or at the conclusion of sanitization or sterilization. Use of the process equipment in subsequent production should start prior to expiration of the CHT.

See Appendix 7 for an illustrative example.

5.3 Clean Out of Place (COP)

Equipment is cleaned out of place as a whole (i.e., tanks) or as a part disassembled from a larger system. The equipment can be cleaned semiautomatically or manually.

COP applications are divided into two groups:

- Automated, for example, parts washers
- Semiautomated, for example, ultrasonic washers

Semiautomated cleaning is automated cleaning with some level of manual involvement, such as disassembly, relocation, cleaning accessory setup, and placement or staging of equipment. The equipment can vary from large vessels to small parts. In general, larger vessels are cleaned internally using cleaning devices (e.g., spray balls or jet sprayer), and small parts are cleaned using an enclosed cleaning apparatus such as a parts washer or ultrasonic cleaner.

5.3.1 COP Stations

Vessel-type equipment can be cleaned using a COP station. The COP stations clean the internal surfaces of equipment. The external surfaces are usually cleaned manually prior to or following the semiautomated cleaning cycle. It is generally preferred to clean the external surfaces prior to cleaning the internal compartment so that internal surfaces do not get re-contaminated during external cleaning.

The manual involvement is usually limited to connecting supply/drain lines and cleaning devices. The COP station can be stationary or mobile. In some cases, a CIP skid can be dual purpose (i.e., CIP circuit and COP).

The advantage of using a COP station is that it is a relatively automated process. Supply and drain line connections can be easily verified. Proper placement of cleaning devices may also not be a concern (depending on equipment geometric shape and internal configuration). If the precise placement of the cleaning device is critical, it should be addressed (e.g., load pattern) during development, prior to cleaning validation, and routinely after validation.

With proper justification (i.e., documented load pattern), validating a COP station can be similar to validating an automated system (i.e., CIP circuit).

Although the cleaning of the external or non-product contact surfaces does not need to be validated, it should be proceduralized and visual cleanliness should be assessed.

5.3.2 Washers

There are various types of washers, for example, parts washers, cabinet washers, and ultrasonic cleaners. Washers have the advantage that they can clean the internal and external surfaces of equipment; however, there are some clear disadvantages. A COP washer example is shown in Figure 5.6.

The placement and staging of the equipment are crucial. The most important aspect when designing and validating the cleaning procedure is part placement and movement during cleaning. Load patterns need to be consistent using pictures of the loaded washer as a guide. Even if performed properly, the equipment can move during cleaning. Layering or stacking parts should be avoided.

For parts directly connected to a port on a cart or directly from a COP skid, unless there is unusual geometry, the equipment should be evaluated and validated as piping.

The placement of equipment cleaned by individual spindles or in a basket needs to be assessed and proceduralized.

When assessing for cleanliness, testing only the final rinse may not be adequate for validation, as the rinse sample is diluted significantly, creating a false sense of cleanliness. In addition, the rinse sample is a compilation of all of the parts cleaned in the load; any failures could not be correlated to a specific piece of equipment. Parts (i.e., worst case) should be sampled individually (i.e., swab or rinse) for cleaning validation.

Figure 5.6: Example of Clean Out Of Place (COP) System on Left and Model of Combined Push-Pull and Rotational Flow through Rectangular Tank on Right
Used with permission from Sani-Matic, Inc., https://sanimatic.com.

5.3.2.1 Cabinet Washers

Cabinet washers are mainly used to clean large to medium vessels. This type of cleaning is similar to COP station cleaning but usually includes exterior surface cleaning. Figure 5.7 has an example of a cabinet washer.

The small parts can also be cleaned using cleaning carts, similar to a parts washer. This type of cleaning should be evaluated similar to a washer.

Figure 5.7: Example of Cabinet Washer System on Left and Rack System Fixtures on Right
Used with permission from Sani-Matic, Inc., https://sanimatic.com.

5.3.2.2 Ultrasonic Cleaners

Ultrasonic cleaners clean using ultrasonic waves. The frequency setting may be fixed or adjustable.

As with washers, part placement and orientation need to be evaluated. During evaluation, the cleaner should be sectioned off into quadrants. Each quadrant needs to be tested to determine the ultrasonic activity, as each quadrant may have a different intensity. The intensity and distribution of the cavitation can be tested using foil strips, kits, or an activity meter. During development and/or validation, the worst-case quadrant can be challenged.

Though ultrasonic cleaners can be very useful in certain cleaning situations, determining the correct temperature, frequency, and optimal cleaning solution can be complex. Because each parameter may play a role, there may be a large initial investment in research and development. For example, higher temperatures may reduce cavitation but some oils may require the higher temperatures. It is important to understand how residue, equipment surfaces, and cavitation respond to temperature, frequency, and cleaning solutions.

Alternatively, ultrasonic cleaners can be used as the first step of a manual cleaning procedure to loosen certain residues, which would mitigate the concern for validating an ultrasonic cleaner.

5.4 Manual Cleaning

Manual cleaning processes continue to play a role in the pharmaceutical industry. Some equipment and parts are not easily adapted for CIP or COP.

Because of the human element, the variability of manual cleaning is the primary concern when validating the cleaning process. The easiest way to address variability is to develop a cleaning process that is more rugged than needed for the product residues, and to clean residues to levels well below the cleaning SL.

Individuals involved in manual cleaning must be adequately trained on the cleaning process(es) [13]. This includes detergents, aqueous utilities, and cleaning aids (e.g., brushes, pads, wipes). Thorough training is also necessary on the individual equipment cleaning procedures. Personnel need to know the extent of disassembly of each piece of equipment to allow adequate cleaning of all parts of the equipment.

Personnel must be familiar with the cleaning process parameters for manual cleaning. While detergent concentration and water temperature might be less critical, the minimum cleaning time for each piece of equipment needs to be followed in order to minimize individual variability as much as possible. Appendix 6 presents a case study on setting parameters for a manual cleaning process.

Qualification of individual cleaning personnel is recommended. Qualification can be done for each equipment group (e.g., tanks) or for a representative complex piece of equipment (e.g., tablet press). Qualification is another way to ensure an adequate and more consistent level of manual cleaning on all equipment. During cleaning validation, different personnel should be used to mitigate the assumption of variability.

Of particular concern is manual cleaning of product residues with low HBEL cleaning limits. For these residues, consistency is critical. A low HBEL cleaning allows no room for error based on variability since a piece of equipment could be visibly clean but still fail the HBEL cleaning limit. In addition to qualified cleaning personnel, implementing frequent monitoring is advisable after cleaning validation is complete.

The validation requirements for a manual cleaning process are the same as for any cleaning process. What differentiates manual processes from CIP and COP processes is the ability to measure the Critical Process Parameters (CPPs) for cleaning. Automated systems routinely record and control temperature, conductivity (detergent related) and time where manual cleaning relies on operator documentation of these parameters.

5.5 Cleaning Parameters and CPPs

The cleaning parameters for a cleaning process include:

- Water quality
- Water temperature
- Water pressure
- Detergent
- Detergent concentration
- Cleaning aids (brushes, wipes)
- Duration (time)
- Force (manual force)
- Initial rinse
- Wash
- Second rinse
- Final Purified Water rinse
- Drying
- Personnel (for manual cleaning)

The CPPs are a subset of these parameters and should be determined during development.

Soil Load

The amount of product residue on the equipment can have an impact on the effectiveness of the cleaning; however, the soil load is not generally considered a CPP for cleaning.

Before cleaning begins, the soil load on the equipment must be defined to ensure consistency in order to better determine the CPPs. To ensure consistent product deposits on equipment, the manufacturing batch record should include steps to recover/remove any remaining batch material from the equipment. For liquid dose equipment this could be as simple as thoroughly draining all equipment. For solid dose equipment, vacuuming and scraping equipment surfaces is often employed, followed by wiping all surfaces with solvent (e.g., 70% IPA) to remove remaining residue, minimizing dust when moving the equipment to the cleaning area, and drying the equipment surfaces.

Residue removal after manufacturing can be subjective but when considered as part of batch reconciliation, a consistent batch yield reflects consistency.

If the batch material removal is completed immediately after manufacturing, there are several advantages: The batch yield is consistently higher; the equipment cleaning personnel are exposed to lower levels of product residue, and the amount of API going into the drain during cleaning is decreased.

The DHT is impacted by the soil residue load and timing of batch material removal. The DHT is much easier to define, defend, and manage when the batch material removal occurs as part of the manufacturing batch record. The DHT begins after manufacturing, so the residue level is much lower and the equipment is dry when a solvent wipe is used. If batch material removal waits until the beginning of cleaning, the DHT will reflect the higher, more variable residue level, and any residual moisture in the residue will dry, potentially making the equipment harder to clean and the DHT as well as the cleaning validation harder to defend.

Water Quality

The water quality is usually based on the local municipal water supply. For cleaning equipment, this is generally satisfactory. The hardness (high mineral content) or softness of the water can impact the effectiveness of detergents. Water quality cannot be controlled for cleaning and is not generally considered a critical cleaning parameter, but the consistency of the local water supply should be monitored by engineering to ensure consistent plant operations.

The quality of water used for the final rinse of the equipment after cleaning should be the same as used for manufacturing (e.g., purified or better).

Water Temperature

Water temperature for CIP systems and some COP systems can be measured and monitored, and automated systems can be programmed to shut down if the water temperature goes outside the programmed range.

Controlling water temperature for manual cleaning is necessary from a cleaning validation and safety perspective. The system should supply water at a certain temperature and then cleaning is validated at that temperature.

Manual cleaning may be conducted under controlled temperature conditions. Installing a temperature gauge in the water line is an option, but unless it is monitored and recorded, its value is limited. There are certain circumstances when cold water works better than hot water for cleaning. An example is some enteric coating components. On the other hand, soft-gel gelatin requires hot water of > 60°C for effective, efficient cleaning.

Establishing water temperature as a CPP for cleaning should be determined during cleaning development. If truly a CPP, then it needs to be measured and controlled. If water temperature is not thought to be critical, then a wide range of temperatures should be considered during development to demonstrate that it is not a cleaning CPP.

Detergent

Note: Other cleaning agents are sometimes used, for example, water, chemicals (e.g., NaOH), or solvents. Detergents are addressed here but appropriate controls should be implemented when other cleaning agents are used.

Alkaline detergents are more effective for cleaning the majority of APIs and excipients. Acidic detergents are more effective for certain excipients; however, neutral detergents can also be effective for cleaning. Since a chosen detergent is a constant for a cleaning process, it would not be considered a cleaning CPP.

Ideally, one detergent is chosen for all cleaning. The goal is to choose a detergent and use a rugged cleaning process to clean the worst-case soil to levels well below the cleaning limit. Use of one detergent simplifies the approach to cleaning and cleaning validation. A low-foaming detergent that is compatible with CIP and COP systems can also be used for manual cleaning and is advisable for the same reasons. If a new worst-case soil is introduced, the use of additional detergents may be necessary, but this should be minimized, since each detergent used requires a separate validation.

If a cleaning process uses two detergents (e.g., an alkaline detergent wash followed by a rinse, followed by an acidic detergent wash or an alkaline detergent wash followed by a rinse, followed by a neutral detergent wash), consider using the dual detergent approach for all products. The dual detergent approach is inherently more rugged and consequently lowers the risk of introducing a new worst-case soil.

Detergent Concentration

The detergent supplier has a recommended concentration (e.g., 2%) and dilution scheme for their detergent. Cleaning development studies, either by the end-user or by the detergent supplier, should confirm the appropriate concentration of detergent that will effectively clean the worst-case product in the facility manufacturing portfolio. A detergent supplier could have literature showing the criticality of the detergent concentration. Otherwise, a range around the recommended concentration can be tested during development to establish whether the detergent concentration is a cleaning CPP.

Cleaning Aids

Manual cleaning is accomplished with the use of cleaning aids. Brushes of different sizes and functions need to be defined and sourced. Examples of brushes include: long and short-handle scrub brushes for cleaning flat surfaces and large and small bottle brushes for cleaning valves and pipes. Non-abrasive, non-shedding scouring pads or wipes can also be used. All cleaning aids must be specifically designated and sourced from the same supplier and used for validation to avoid questions about the effectiveness of the cleaning process. If the cleaning aid is used consistently, it is not a CPP. However, if different cleaning aids are used or are not specifically identified in the cleaning procedure, they might be considered critical.

The determination to designate cleaning aids as single-use or reusable is a major decision. Single-use is easier to understand and defend. To reuse cleaning aids, cleaning, drying and storage must be defined. The number of times the aids can be reused needs to be defined and justified. The use of the cleaning aids must be tracked and documented.

Initial Rinse Technique/Time

Note: The timing of rinse and cleaning steps should be considered CPPs and captured individually or as a group on a case-by-case basis.

The soiled equipment is initially rinsed with water or solvent following a top to bottom pattern to remove any loose residue. The length of the rinse should be defined during development, and measured and documented during validation.

Equipment Cleaning Technique/Time

The equipment is washed with the prepared detergent solution per the equipment SOP. CIP and COP systems have cleaning programs for the equipment. Manual cleaning should use a top to bottom or inside to outside pattern and overlapping strokes. The length of the wash should be defined during development, and measured and documented during validation.

The level of detail should be enough to ensure consistent cleaning. Added detail is appropriate for those pieces of equipment requiring more specific instructions based on their complexity or known history of cleaning problems.

Second Rinse/Time

The cleaned equipment is rinsed with water following a top to bottom pattern to remove any remaining residue and the detergent. The length of the rinse should be defined during development and measured and documented during validation.

Final Purified Water (PW) Rinse/Time

The rinsed equipment is final rinsed with PW for better results following a top to bottom pattern to the rinse water from the equipment. The grade of water used for the final rinse reflects the grade of water used for manufacturing. This prevents dried water residue marks on the equipment. The length of the rinse should be defined during development, and measured and documented during validation.

Drying

The cleaned equipment can be dried using a drying room, compressed air, or solvent (e.g., 70% IPA) to remove remaining PW from the equipment. Equipment can also be left to air dry, which takes longer. If solvent is used, it takes about 15 min for the solvent (e.g., IPA) to completely evaporate. The equipment surfaces should be inspected as clean and dry prior to covering and storing. Drying is not considered a cleaning CPP as long as the equipment is dry.

Documentation

All CPPs for a cleaning process should be recorded. Some cleaning parameters are outside the cleaning area control, including the water quality and hot water temperature. The lot and expiration date of the detergent should be recorded along with the routine preparation of the working detergent.

Recording times for rinsing and washing can range from recording the timing for each part, to recording one time for the entire piece of equipment. The greater the level of detail, the better the demonstration of consistent control of the cleaning procedure. Each facility must decide the level of detail they can record to demonstrate reliable, consistent equipment cleaning without adversely impacting cleaning activities.

5.6 Worst-Case Products

Product grouping is a way to reduce validation activities in sites with multiple products and processes. Products may be grouped together if they are cleaned by the same process. If a product in that group requires a more aggressive cleaning process, that product becomes the worst-case product in that group. All products in that group should be cleaned using the worst-case products parameters.

If the cleaning process has yet to be determined, group products by type and determine the worst-case product by performing a risk assessment considering solubility, ADE/PDE levels, and cleanability. Each group of products may have a different cleaning process. They may be kept as different processes; however, future errors may be reduced or avoided if one cleaning process using the worst-case product residue requirements is chosen.

The worst-case product is a combination of the product residue that is the hardest to clean and the lowest cleaning limit for the products manufactured at a facility.

The hardest-to-clean formulation can be assessed in a number of ways. Formulations can be assessed for cleanability based on the physical properties of the formulation components. The assessment looks at the physical properties of all formulation components including solubility in water and their composition percentage to determine which formulations are considered hardest to clean. Operator cleaning experience regarding hard-to-clean formulations is subjective but often correct. Laboratory coupon cleanability studies can be conducted on several candidates for worst-case to arrive at the hardest-to-clean product(s) and recommended cleaning conditions for the product. Once the hardest-to-clean residue from a risk assessment has been determined, design the cleaning process based on the removal of that soil. If the hardest-to-clean product can be cleaned down to the level of the product with the lowest acceptance criteria, then all products with the common cleaning process are considered validated.

Equipment grouping strategies (also referred to as family approaches) are often used to simplify aspects of cleaning validation. Establishing through scientific rationale that equipment sharing the same design and construction can be grouped for validation purposes may reduce the total number of validation runs necessary to demonstrate consistency of the cleaning process. For example, five 500 L formulation tanks with the same dimensions can be grouped for validation purposes. Although all pieces of equipment are cleaned during validation, less runs are needed to complete cleaning validation.

The basic purpose of product grouping determines the representative or worst-case products manufactured on a particular piece of equipment or in an equipment train. Assurance that the cleaning method removes those products to an acceptable level can also provide assurance that the cleaning procedure removes residues of the other products within that group to the same or better level. A single validation study utilizing a worst-case approach can then be carried out, which takes account of critical issues.

These approaches are supported by PIC/S [23] and others. For cleaning validation purposes, cleaning procedures for products and processes that are very similar can be grouped together with proper justification, and may not need to be individually validated. It is considered acceptable to select a representative range of similar products and processes [4]. Strategies that could be followed include grouping by product or grouping by equipment [28].

5.6.1 Residue Types

Residues can have various characteristics or conditions that may affect the chosen cleaning process. Residues may be classified as easy to clean in water or very soluble in water; too easy to clean and highly mobile in liquid state; moderately easy to clean; having viscosity issues too difficult to clean, oily substance, builders or excipients; hard to clean such as denatured proteins, dyes, titanium dioxides, and thin film formers.

The condition of the soil may impact the cleaning process. Typical conditions are wet, dry, steamed, baked, and compacted. As residues dry on the surface or are baked on the surface through jacketed vessels, they may become harder to clean.

It is important to model laboratory evaluations after processing conditions with respect to residue conditions and amount on the surface. Ensure worst-case conditions are tested during the laboratory evaluation. Cellulose-based products become harder to clean as they dry whereas denatured proteins and polymers are harder to clean if they are heated and baked onto the surface. For example, if the process residue is wet granulation, ensure the product is reconstituted with the appropriate concentration of solvent prior to testing since dry wet-granulation is easier to clean than when re-wetted and applied to the surface.

5.6.1.1 Soil Residue Load

High levels of the residue may saturate the cleaning solution. This may be due to a large surface area cleaned using a low volume to surface area ratio of cleaning solution, or from the level of soil remaining on the surface per surface area. In these instances, an initial rinse step may be required or higher volumes of wash solutions used. The typical volume of cleaning solution to use is approximately 20%–30% wash solution compared to total hold-up volume of the equipment. Additionally, a single-pass wash step versus a recirculated wash step, at least initially, may aid in removing gross soil from the equipment surface. Higher residue levels may also be encountered on equipment surfaces due to design issues such as splashing above the liquid level line from the lack of, or use of, an inappropriately sized dip tube; use of reducers either restricting flow or causing a reduction in flow rate; or j tubes directing flow above the liquid level line, horizontal surfaces, and residue located along the air-liquid interface.

5.6.1.2 Materials of Construction

The MOC should be considered as part of the cleaning process development. Some residues may be more difficult to clean from certain surfaces due to surface interaction, roughness, or porosity, as in the case of some plastics. The MOC should be assessed to determine if they are compatible with the cleaning agent and temperature.

5.6.2 Laboratory Evaluation/Confirmation

A laboratory evaluation will help determine the starting cleaning parameters of a product from an equipment surface using representative surface or coupons to base the initial cleaning trial on during the design phase (Figure 5.8). A laboratory evaluation or coupon study may also be used to optimize a current cleaning process, to establish corrective action in the event of a cleaning failure, or as a worst-case product determination. The evaluation should provide a cleaning recommendation at normal operating range and an understanding of the design space or proven

acceptable range. The laboratory study should simulate the residue amount, manufacturing conditions, and cleaning method. The evaluation will determine which cleaning agent to use along with optimal cleaning parameters such as how long to clean the equipment, temperature to use for wash and rinse steps, and concentration. DHTs should also be assessed as part of this evaluation.

Figure 5.8: Image of a Stainless Steel Coupon used for a Laboratory Cleaning Evaluation
Used with permission from STERIS, www.steris.com.

A laboratory evaluation starts by selecting coupons to match the MOC found in the manufacturing process; however, testing should initially be performed on the predominant MOC (e.g., SS) to assess if visually clean. Ensure coupons are thoroughly cleaned and if stainless, passivated if necessary. Cleanability is assessed by coating coupons with the product in a manner where the amount of residue per surface area is controlled and recorded. The product is then conditioned on the coupon to simulate equipment surface conditions during the process including the DHT. The cleaning process is then screened initially in a beaker to test various conditions that may be optimized or assessed in other representative systems. An overview of a laboratory evaluation is provided in Table 5.1.

Table 5.1: Overview of a Laboratory Coupon Evaluation

Step	Activity
1	Weigh coupon on an analytical balance to obtain the pre-coating weight.
2	Coat coupon with product sample.
3	Air dry, bake, or compress the soil onto the coupon (~100 cm^2).
4	Weigh the soiled coupon on an analytical balance for the post-coating weight.
5	Soiled coupons are washed using one or more cleaning methods such as agitated immersion (beaker study), spray wash, manual scrub using a nylon brush, or cascading flow.
6	Remove the coupon from the cleaning solution and visually observe for cleanliness.
7	Rinse coupon while examining as it drains down for water break-free surface.
8	Dry and weigh on an analytical balance for the post-cleaning weight.

If performing a gravimetric assessment, a tare weight should be documented prior to coupon soiling along with recording the weight of the coupon once the product has been applied. Coupons are then coated with the residue for a specific time, temperature, and conditions, and then held under the conditions consistent with the DHT conditions. After the coupon has been exposed to residue and soiling conditions, it may be cleaned by various cleaning methods. An agitated immersion test is a relatively easy experiment to determine if the cleaning agent is appropriate. It consists of placing the coupon in a beaker of cleaning solution at various temperatures, concentrations, and time. Other types of cleaning action, including impingement, cascading, or scrubbing can also be tested in coupon studies. Several detergent suppliers will assist with these types of studies.

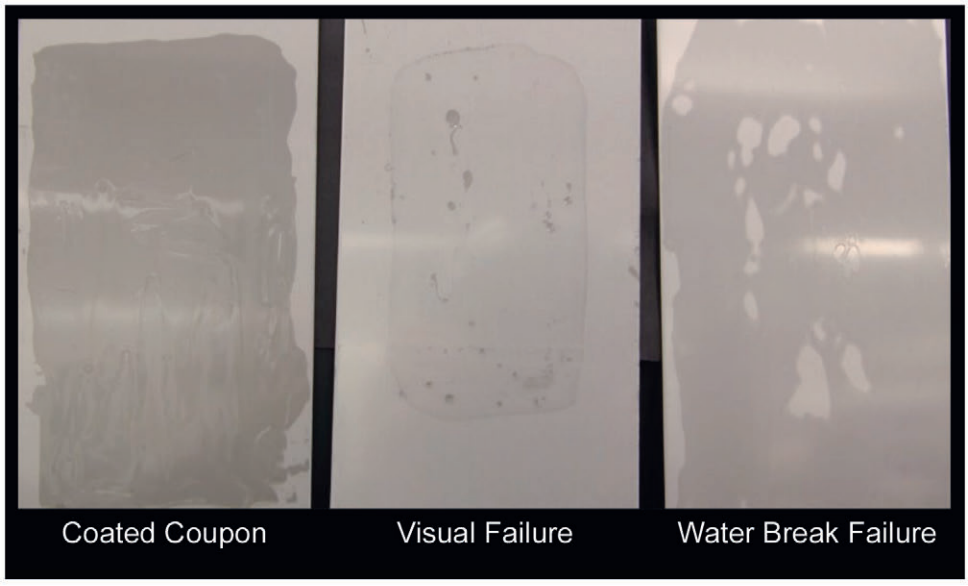
The coupon should be visually inspected and either returned to the cleaning agent for additional time or evaluated for cleanliness using more sensitive methods. A visually clean coupon is rinsed and assessed for cleanliness by visual examination, performing a water break test and a final gravimetric assessment (Figure 5.9).

The visual examination of the coupon surface is not meant to ensure levels below a determined visually clean threshold, which may still be greater than the analytical test methods used for cleaning validation. The visual examination is a qualitative method to assess if there is any remaining residue on the coupon. The advantage of the visual examination when used as criteria for a coupon evaluation is the ability to view under various lighting conditions, angles, and under both wet and dry conditions.

The Water Break Test Method, from ASTM A380 [50], is used to test for the presence of hydrophobic contaminants on a cleaned surface. The contaminated area has a lower surface tension than water, which causes water to bead up at the soiled location. The test method is a rapid, non-destructive test used only for items that can be dipped.

When the coupon is visually clean, rinse it with deionized water for approximately 10 s in a vertical orientation. The surface is examined as the film of water drains. If the surface is clean, water will form a thin, continuous film. Let the coupon dry and inspect the dry surface for visual failures and obtain a post-clean weight. A clean coupon for gravimetric assessment can be confirmed down to ± 0.0001 g with an analytical balance. A large coupon is considered 100% clean by weight if its pre and post-cleaning weights are < 0.1 mg from each other. The coated coupon surface is approximately 100 cm^2 for wet samples and approximately 200 cm^2 for dry samples. The coated surface and sensitivity of the balance corresponds to < 0.5 $\mu g/cm^2$ or 1 $\mu g/cm^2$ assuming a uniform distribution.

Figure 5.9: Images of Coupons from a Laboratory Study
Used with permission from STERIS, www.steris.com.



5.6.3 Equipment Dirty Hold Time (DHT)

Worst-case hold times should always be evaluated during cleaning validation studies, but this can be mitigated if there is information from product development that the nature of the soil does not change during extended holds. Typically, moist soil becomes more difficult to remove when dry. The total DHT should be evaluated as opposed to the time it takes to dry onto the surface, since what appears dry is not always completely dry. The soil may undergo environmental changes during the elapsed time, which should be considered during validation. For example, in oral solid dose manufacturing, dry powders may absorb moisture and cake or harden, becoming more difficult to clean; conversely, some soils may be easier to clean after time.

There is no standard duration for typical DHT. Consult with the Operations Group to determine the optimal and achievable DHT. The length may be schedule driven where equipment may not be able to be committed for validation purposes for an extended length of time. DHT duration may range from 1 hour to 30 days (which is extreme), with 3 to 7 days being typical since potential contamination risk increases with time. Microbial proliferation and equipment condition should also be considered when establishing DHTs.

Once DHT is validated, the equipment must be cleaned prior to exceeding the DHT limit. If the equipment DHT limit is exceeded for any reason, the equipment requires verification that it meets the established acceptance criteria outlined in the original cleaning validation protocol. This may be a onetime verification test with reduced testing to ensure the equipment is back to baseline.

When possible, establish DHTs in increments greater than 24 hours. This allows for the end-user to calculate the delta dirty hold in days rather than in hours, which may minimize calculation errors and verification testing if DHTs are exceeded when in operation.

Consider all process equipment used in the equipment chain, and establish DHTs so all pieces of equipment in the operation are able to be cleaned in the allotted amount of hold time due to resource constraints, whether they be operators, utilities, or number of CIP skids.

5.7 Detergents

The term detergent is commonly used to describe an aqueous-based cleaner formulated with functional components to enhance certain mechanisms of a solution over water or commodity cleaning agents. These solutions are generally referred to as formulated detergents and are blended to combine the most robust solution for specific applications. Further, certain characteristics are considered when formulating such as analytical detection, rinsability, material compatibility, antimicrobial effectiveness, and foam characteristics. While the cost as purchased per kilogram may be higher for the formulated cleaner, the overall process cost may be less due to time savings [51].

Formulated detergents consist of water, which serves as a polar solvent, and may also contain, but not limited to, a blend of bases, acids, surfactants, dispersants, chelating agents, solvents, and oxidizing agents. Surfactants, or surface active agents, orient themselves at surfaces or form micelles that provide a mechanism for solubilization, emulsification, and dispersion. Surfactants are structures with water-insoluble and water-soluble elements and are ordered based on the charge of the polar or water-soluble head. Anionic surfactants contain a negative charge on the polar head, which is important for dispersion and detergency characteristics in aqueous-formulated detergents. Nonionic surfactants contain no charge and are compatible with anionic and cationic surfactants. Nonionic surfactants tend to work exceptionally well as emulsifying agents for immiscible liquids. Cationic surfactants carry a positive ionic charge usually associated with nitrogen in an amine or quaternary ammonium structure, and amphoteric surfactants may become positive or negative on the polar end based on the pH of the solution.

It is recommended to use alkaline chemicals for protein and organic soils and acidic chemicals for inorganic, mineral-based soils [52]. Formulated acidic cleaners are routinely used for derouging and passivation applications. Use of formulated detergents may be advantageous over water, solvents, or commodity cleaning agents such as sodium hydroxide alone since they may be more effective in cleaning a broader range of soils and products encountered in pharmaceutical and biopharmaceutical manufacturing.

5.8 Inactivation and Denaturation

For multiproduct cleaning validation, the conventional approach for setting an acceptance limit for the process residue is based on the MACO of the API (depending on the process soil, API refers to the active pharmaceutical ingredient in the DP, DS, or DS intermediate). However, if the API becomes pharmacologically inactive during cleaning the acceptance limit does not need to be based on active product. This is an important consideration in biopharmaceutical manufacturing because the cleaning conditions are generally aggressive enough to inactivate the product.

The experimental approach and analytical methods for assessing inactivation of the API during cleaning are described in this section. A rational approach for setting safety-based acceptance limits for inactivated product and process residuals is described in Section 6.2.

The scope of this section is limited to biopharmaceutical cleaning processes; nonetheless, the underlying concepts may be useful in designing inactivation studies and setting acceptance limits for other types of pharmaceutical cleaning processes.

5.8.1 Limitations of the Conventional MACO Approach

An important regulatory expectation for multiproduct cleaning validation is to demonstrate that potential carryover of the previously manufactured API (Product A) into the subsequently manufactured product (Product B) is below an acceptable level. This criterion is often assessed through a MACO calculation for the previously manufactured API. The MACO calculation is typically based on the ADE of the previously manufactured API [3, 48, 53, 54, 55].

A limitation of the conventional MACO approach is that it assumes that the product is active after cleaning. This has important implications for biopharmaceutical manufacturing because the API is often inactivated by the cleaning process [56, 57].

Another limitation of the conventional MACO approach is that the calculated acceptance limits are often below the LOQ of non-specific analytical methods, such as TOC. The LOQ of TOC-based methods is typically between 0.05 and 0.2 ppm. The large surface areas and small batch sizes involved in biopharmaceutical manufacturing further exacerbate this issue. Product Specific Immunoassays (PSIA) such as ELISA and EIA have been used to address this issue; the LOQ of most PSIAs is on the order of 10 ppb. PSIAs detect activity indirectly by recognizing specific epitopes (short sequences of amino acids) in the API; however, epitopes are known to degrade during cleaning, and thus the results can be misleading [13, 58].

Other limitations of the conventional MACO approach are discussed in the literature [58].

5.8.1.1 Product Inactivation Approach

With the product inactivation approach, if the API is inactivated during cleaning, the acceptance limits may be set based on the inactivated product instead of the API. The product inactivation approach is therefore more reflective of the phenomenological aspects of the cleaning process. Additionally, the acceptance limits based on inactivated product are very unlikely to be below the LOQ of TOC. In fact, inactivated product would have no pharmacologic activity and be more similar to an excipient with a cleaning limit of visually clean. Thus, the product inactivation approach alleviates the limitations of the conventional MACO approach.

However, with the inactivation approach, there must be documented evidence that the product is completely inactivated and that the inactivated product fragments are not pharmacologically active. If there is any intact product remaining, the MACO approach still needs to be applied. Although with a mostly inactivated product, there is a lower risk of failing a specific residue assay for cleaning.

The methodology described in this section includes experimental simulation of the cleaning processes at small-scale and analytical methods to evaluate inactivation of the API during cleaning. A rational approach for setting safety-based acceptance limits is described in Section 6.1.3.

Proposed Methodology

Inactivation of the product during cleaning has important implications for cleaning validation of multiproduct equipment. If it can be demonstrated that the product becomes pharmacologically inactive during cleaning, there is limited value in verifying the removal of the active ingredient. Instead, it is more appropriate to demonstrate that the inactivated product has been removed below a defined acceptance limit. This is consistent with the expectation that the carryover of an extrinsic impurity into a subsequent batch should be justified from the standpoint of the safety and efficacy of the product. It also obviates the need to develop PSIAs for cleaning validation.

Biopharmaceutical cleaning cycles are generally designed to expose product contact equipment to extreme pH (< 2 and > 13) and high temperature (60°C–80°C) for several minutes. Under these conditions, monoclonal antibodies, therapeutic proteins, and other biopharmaceuticals are known to degrade and denature rapidly and are therefore likely to become pharmacologically inactive [56, 57]. The product inactivation approach should therefore be considered for biopharmaceutical cleaning validation.

Guidance for Designing Inactivation Studies

Fragmentation and inactivation of an API during cleaning can be assessed by exposing the process soil to worst-case cleaning conditions at bench scale [59, 60]. The results of the bench-scale studies can justifiably be extrapolated to the full-scale cleaning process. This is because under worst-case cleaning conditions of laminar flow and low shear rate fragmentation and inactivation are independent of scale (i.e., they depend on cleaning parameters that are not a function of the spatial coordinates of the system, such as time, temperature, concentration, and the ratio of cleaning solution to process soil).

For glass or glass-lined vessels, the bench-scale experiments are typically performed in a glass vial or dialysis cassette. For SS equipment an appropriate small vessel could be used. The bench-scale studies are designed to simulate full-scale cleaning conditions that are least conducive (worst case) for inactivation. For example, for a chemical wash, the lowest applicable concentration of cleaning agent, temperature, duration, and ratio of cleaning solution to residual process soil should be considered in simulating the cleaning cycle at bench scale. Other operating parameters that can contribute to product inactivation include DHT and associated drying conditions (humidity and air circulation rate), and shear rate due to impingement and turbulence.

An operating parameter or step can be eliminated from the experimental design if its elimination represents a worst-case scenario from the standpoint of inactivation. This approach can be leveraged to simplify the bench-scale studies. For instance, if it is reasonable to assume that product inactivation increases with shear rate, then it can be eliminated from the experimental design (i.e., the shear rate need not be simulated in the experiment). Similarly, the ratio of cleaning solution to process soil can be reduced, and the acid wash and rinse steps can be eliminated to minimize dilution of the process soil, and facilitate detection of the process residue in the sample. When making such changes, unexpected effects such as aggregation of the API can occur. It is therefore important to make sure that the modifications do not result in experimental artifacts.

If the cleaning cycles are being developed or modified, the inactivation study should be designed to evaluate the effect of key operating parameters on the fragmentation and inactivation rate of the API. This information, together with data from cleanability studies [61, 62], can be used to identify cycle parameters that are effective in inactivating the API.

For existing cleaning cycles, the cleaning conditions for the inactivation study should be based on worst-case operating parameters for all systems involved. For instance, if different systems are cleaned with different cleaning solutions and at different temperatures, then the study should be performed with the mildest cleaning solution, at the lowest cleaning agent concentration, and the lowest temperature, if these conditions are least conducive for inactivation. Further, for CIP systems with multiple toggle paths or circuits, the duration of cleaning should be based on the toggle path with the shortest cleaning time.

As an example, after exposing some process soils to worst-case cleaning conditions, the samples are titrated to a neutral pH, and cooled to 4°C to minimize further fragmentation and inactivation of the API. The samples are then subjected to analytical testing as described in the Section 5.8.2.

If the results indicate that the API is not inactivated during cleaning, then the acceptance limits should be set based on the acceptable carryover of the API [3, 48]. If the API is partially inactivated, then where possible, the acceptance limits should be determined for the API, as well as for the inactivated product, and the lower of the two limits should be used. Alternatively, the cleaning parameters can be modified to ensure inactivation of the API. This can be facilitated by running additional studies to characterize the effect of specific cleaning parameters on the API.

5.8.2 Recommended Analytical Methods to Evaluate Fragmentation and Inactivation of API

Analytical methods commonly used to evaluate the effect of cleaning parameters on the previously manufactured API are described in this section. These methods are used to evaluate fragmentation and inactivation of the API at bench scale, and to detect target impurities (in this case, the previously manufactured API and/or inactivated product in the process residue) in cleaning validation samples. The analytical results are used to understand the impact of the cleaning conditions on the process soil, and to set rational safety-based acceptance limits for the target impurities. The detection methods are used to verify that the concentrations of target impurities in cleaning validation samples are below their respective acceptance limits.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) or Capillary Electrophoresis (CE) are generally used to characterize *fragmentation* of the API during cleaning. For SDS-PAGE, a 4%–20% gradient corresponds to a Molecular Weight (MW) range of 4 to 250 kDa, which is sufficient for most biological APIs. While CE provides greater sensitivity, lower variability due to the absence of staining, and high throughput capability as compared to SDS-PAGE, both methods are adequate for demonstrating distinct, size-based separation of fragmented protein. Size Exclusion High Pressure Liquid Chromatography (SE-HPLC) can also be utilized for size-based separation of protein fragments; however, it can be difficult to obtain a distinct size-based separation across a wide range of fragment sizes.

Inactivation of the API can be evaluated by methods that measure loss of biological activity or function (binding sites that are functionally intact), such as a bioassay. These methods measure the relative amount of biologically active product. Thus, they can be used to evaluate the degree of inactivation of the API during cleaning.

With the above methods, appropriate standards and untreated controls should be included to provide a basis for comparison, and to assess the impact of any experimental artifacts and potential matrix effects. For SDS-PAGE, appropriate MW markers should be included to facilitate comparison of the fragments to the untreated controls.

6 Acceptance Criteria

Acceptance criteria for a cleaning process performance qualification (CPPQ) consist of several elements:

- Meeting safe residue limits
- Meeting criteria for visually clean
- Demonstrating a reproducible and consistent cleaning process
- Demonstrating control of bioburden and endotoxin levels

Table 6.1 provides a summary of the historical evolution of guidance for establishing residue limits for cleaning processes. The establishment of cleaning residue limits based on HBELs is the current and appropriate approach to use. Refer to Section 6.1 for more details.

Table 6.1: Historical Review of Cleaning Validation Limits Guidance

Year	Reference	Cleaning Residue Limit Approach
1993	Fourman, G. and Mullin, M, "Determining Cleaning Validation Acceptance Limits for Pharmaceutical Manufacturing Operations," *Pharmaceutical Technology* [48]	Any carryover of product residue shall meet the following criteria: No more than 0.001 (a factor of 10 because pharmaceuticals are often considered non-active at 1/10th of the dose, 10 to ensure the cleaning program is robust and the final 10 as a safety factor) dose of any product will appear in the maximum daily dose of another product. No more than 10 ppm of a product will appear in another product. No quantity of residue visible on the equipment.
1993	FDA Guide to Inspections Validation of Cleaning Processes [17]	Basis for limits must be scientifically justifiable. Mentions as examples 10 ppm, 1/1000 of the normal therapeutic dose (biological activity). Surfaces should be visibly clean.
1998 (Revised 2012)	PDA Technical Report No. 29 "Points to Consider for Cleaning Validation" [37]	Numerical limits should have a logical and scientific basis. Safety factors used based on the type of products and risk. Fractions of therapeutic dose for Topical Products (1/10th to 1/100th), Oral Products (1/100th to 1/1000th), Parenteral and Ophthalmic products (1/1000th to 1/10,000th), Investigational products (1/10,000th to 1/100,000th).
2000	ICH Q7 Good Manufacturing Practice for Active Pharmaceutical Ingredients [21]	*"Limits can be established based on the minimum known pharmacological, toxicological or physiological activity of the API or its most deleterious component."*
2010 (Revised 2017)	*ISPE Baseline® Guide: Volume 7 – Risk-Based Manufacture of Pharmaceutical Products [Risk-MaPP] (Second Edition)* [3]	Residue limits based on Health-Based Exposure Limit (HBEL) such as Acceptable Daily Exposure (ADE) values.
2014	EMA: Guideline on setting health based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities [11]	HBEL (Permitted Daily Exposure (PDE)) through the derivation of a safe threshold value should be employed to identify the risks of cross contamination in shared facilities. Alternate approaches could be accepted if adequately justified.

Table 6.1: Historical Review of Cleaning Validation Limits Guidance (continued)

Year	Reference	Cleaning Residue Limit Approach
2015	EudraLex GMP Annex 15: Qualification and Validation, Section 10 Cleaning Validation [4]	Based on toxicological evaluation. References EMA 2014 Guidance on setting HBEL.
2015	PIC/S Guide to Good Manufacturing Practice for Medical Products, Annex 15 [9]	Limits for carryover should be based on a toxicological evaluation of the active materials. References EMA 2014 Guidance on setting HBEL.
2018	EMA: Questions and answers on implementation of risk-based prevention of cross-contamination in production and 'Guideline on setting health-based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities' [7]	Answers commonly asked questions regarding EMA 2014 Guideline. *"HBELs should be established for all medicinal products."* *"For existing products, manufacturer's historically used cleaning limits should be retained and can be considered alert limits provided that when taking cleaning process capability into account, they provide sufficient assurance that excursions above the HBEL will be prevented."*
2020	PIC/S: Questions and Answers on Implementation of Risk-Based Prevention of Cross-Contamination in Production and 'Guideline on Setting Health-Based Exposure Limits for Use in Risk Identification in The Manufacture of Different Medicinal Products in Shared Facilities' [27]	Adoption by PIC/S of the EMA approach.
2020 Draft	WHO: Draft Working document QAS/20.849, Points to consider on the different approaches – including HBEL – to establish carryover limits in cleaning validation for identification of contamination risks when manufacturing in shared facilities [29]	This draft document incorporates HBELs as part of the baseline approach to setting cleaning limits.

The criterion of visually clean is the most common criterion used for routine cleaning and a common regulatory expectation. Refer to Section 6.3 for more details.

The consistency of a cleaning process can be assessed by evaluating the cleaning process capability and monitoring the cleaning performance. Refer to Section 6.4 for guidance on how to assess process consistency and establish control limits to monitor and control the process after CPPQ.

It is important to measure and document the natural or normal level of bioburden in the equipment to be cleaned. This will help in establishing appropriate bioburden controls to prevent microbial proliferation. Refer to Section 6.5 for guidance on how to establish bioburden controls.

6.1 Cleaning Residue Limits for Shared Facilities

Equipment should be cleaned to a level where any retention or carryover (API, process intermediates, excipients, impurities, by-products, degradants, accumulated product, and cleaning agents) from previous processing does not alter the safety, identity, strength, quality, and purity of the next product manufactured. In consideration of this, there are two important aspects when determining the level of required cleanliness of equipment (product contact surfaces):

- The equipment must be visibly clean.
- The equipment must be cleaned to levels lower than the SL [11].

A safe residue limit, or SL represents the acceptable cleaning limit corresponding to a safe amount of residue in the next product dose (i.e., DP) or batch (i.e., DS).

A main component of the SL formula is the Maximum Allowable Carryover (MACO).

6.1.1 Determining the MACO[5]

The MACO is expressed as either the carryover concentration or mass of carryover of contaminates allowed in the next manufactured batch from a patient safety standpoint. (When expressed in terms of concentration, it must be multiplied by the smallest next product batch size to determine the amount of carryover.)

Because the MACO is used to calculate the SL, it is critical that each company select the most appropriate MACO formula. This is done by considering the characteristics or properties of the active components (e.g., DS, DP). Table 6.2 provides a summary of the applicability of MACO approaches.

Table 6.2: Approaches to MACO Determination

MACO Approach	Applicability	Refer to
ADEs and PDEs	• Actives • Cleaning agents • Degraded and/or denatured actives and protein fragments[1] • IMPs • Chemical (non-API)	Section 6.1.1.1
Threshold of Toxicological Concern	• IMPs[2] • Genotoxic actives with insufficient toxicological data available for an ADE or PDE • Degraded and/or denatured actives and protein fragments • Cleaning agents • Chemical (non-API)	Section 6.1.1.2
Notes: 1. Scientifically justified R&D methods may be used to assess inactive fragments of human therapeutic proteins, either from API or product manufacturing. 2. See Section 9.7 for further information.		

[5] With the introduction of safe cleaning limits based on HBELs, the MACO term should be considered a Maximum Safe Carryover (MSC), which is the maximum amount of carryover of a residual process residue (API, cleaning agent, degradant, and so forth) into the next product manufactured without presenting an appreciable health risk to patients [12]. For simplicity, this guide has kept the terms MACO or Safe MACO to denote material carryovers from one product batch to the next product on shared equipment.

6.1.1.1 ADEs and PDEs

Acceptable cleaning residue limits should be based on toxicological assessments using HBELs (i.e., ADE or PDE) as a baseline. ADE represents a dose that is unlikely to cause an adverse effect if an individual is exposed, by any route, at or below this dose every day for a lifetime. ADEs and PDEs are effectively synonymous per EMA [11], and PICs [25] guidelines. Therefore, establishing cleaning residue limits based on HBELs is the appropriate approach to use when calculating a safe maximum carryover. Accordingly, a cleaning limit based on an ADE or PDE value is used as the safe residue limit criteria for CPPQ.

Determination of PDE values involves qualified experts in relevant areas (e.g., toxicology, pharmacology) and requires the evaluation of hazards, critical effects, no-observed-adverse-effect-levels (NOAEL) for findings considered critical effects, and the use of appropriate adjustment factors. The derivation of PDE values also considers the intended clinical route of administration and corresponding bioavailability[6] [11].

6.1.1.2 Threshold of Toxicological Concern (TTC)

TTC, which can be considered a precursor to a HBEL, are generally accepted by regulatory bodies for determining the MACO when there is only preliminary toxicological data available, such as IMPs, cleaning agents, or genotoxic active substances with no discernible threshold. (See Table 6.2 and *ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3].) There are four main scientific articles that discuss TTC application, Dolan et al. [63], Bercu and Dolan [64], Kroes et al. [65], and Munro et al. [66].

As sufficient toxicological data becomes available to establish an ADE or PDE, an evaluation should be performed to recalculate the SL using the ADE or PDE.

6.1.1.3 Therapeutic Dose

The therapeutic dose approach is a legacy approach that was used prior to the paradigm shift to justify cleaning limits using HBELs (see Table 6.2). They are determined by dividing the minimum daily therapeutic dose by a safety factor (i.e., usually 1000, but can vary depending on route of administration). The concept is that a fraction (e.g., 1/1000) of the minimum daily therapeutic dose would have no therapeutic effect in an individual and therefore ensure patient safety.

A therapeutic dose is not directly related to the safety of the DS, but rather to the efficacy of the DS. It does not consider adverse effects or length of exposure (i.e., ranging from a onetime exposure to a lifetime of exposure). Large safety factors are applied to ensure patient safety. The result is that therapeutic dose-based limits are generally lower in comparison to ADE and PDE-based limits.

For organizations with legacy cleaning limits based on the therapeutic dose approach or distributing to countries where the therapeutic dose is the regulatory basis for setting cleaning limits, determining SLs using an ADE or PDE is necessary. Organizations need to identify whether the cleaning residues would present a risk of causing adverse health effects in exposed individuals. If the legacy limit based on a therapeutic dose is lower than the limit calculated using the ADE or PDE values, the legacy limit may be used as an alert level in the cleaning program. If the ADE/PDE derived limit is lower than the existing legacy limit based on therapeutic dose, then the new SL based on ADE/PDE is implemented. The implementation of the new limit requires assessment for impact to the established cleaning validation and cleaning process.

[6] Per the EMA Guide [11] *"While the PDE value derived for an active substance (contaminant) generally is based on studies applying the intended clinical route of administration, a different route of administration may be applied for the active substance or medicinal product subsequently produced in the shared facility. Changing the route of administration may change the bioavailability; hence correction factors for route-to-route extrapolation should be applied if there are clear differences (e.g. > 40%) in route-specific bioavailability."* These extrapolation factors are considered as part of the PDE value calculation when needed and may result in higher PDA values.

6.1.2 Drug Substance and Drug Product MACO Calculations

Below shows the two main ways to calculate the MACO.

MACO Approach	Calculation
HBEL	$\frac{\text{PDE or ADE (mg/day)} \times \text{MBS (mg)}}{\text{MDD or STDD (mg/day)}}$

MACO Approach	Calculation
TTC	$\frac{\text{TTC (mg/day)} \times \text{MBS (mg)}}{\text{MDD or STDD (mg/day)}}$

Where:

Abbreviation	Term	Definition
MBS	Minimum Batch Size	The smallest batch that will be manufactured on the equipment. It is the batch following manufacturing of the batch being cleaned.
MDD	Maximum Daily Dose	Used when calculating MACO for Drug Product (DP). **Note:** The units for this term can be in: tablets, ml, mg of API, mg of tablet; but the units of the MDD and MBS must be the same for the calculation.
STDD	Standard Therapeutic Daily Dose	Used when calculating MACO for Drug Substances (DS). It is the standard therapeutic dose prescribed (i.e., mg of active ingredient).

6.1.3 Determining Cleaning Residue Safety Limits

Once the MACO is calculated, it is used to calculate the swab or rinse sample SL. Because there are different sampling strategies (i.e., swab sampling, swab and rinse sampling, and single equipment rinse sampling), SL formulas have various forms. Refer to Appendix 1 for detailed SL calculations using examples. An example is also shown in Appendix 2.

In general:

$$\text{Safety Limit (µg/ml)} = \frac{\text{MACO (µg)} \times \text{SSA (cm}^2\text{)}}{\text{TSA (cm}^2\text{)} \times \text{DV (ml)}}$$

Where:

Abbreviation	Term	Definition
MACO	Maximum Allowable Carryover	Maximum allowable mass of cross-contamination or contamination
SSA	Sample Surface Area	Equipment surface area to be sampled
TSA	Total Surface Area	Total direct product contact surface area of equipment or equipment train that can contribute to cross-contamination or contamination. For shared equipment, it includes all equipment shared between actives or products.
DV	Dilution Volume	Desorption volume (i.e., swab samples) or rinse sample volume*
*Rinse samples can be taken either during the final rinse or as a separate rinse following the cleaning cycle.		

The cleaning residue SLs justified by HBELs are not intended to be used as the routine cleaning limits after the CPPQ. The objective is to establish the cleaning process in such a way that it operates with sufficient controls to prevent residues from exceeding the SL. This can be achieved by establishing process control limits based on the process variability and capability.[7] Refer to section 6.4 for guidance on setting process control limits for the qualified cleaning process.

6.1.4 Rinse and Swab Safety Limit Calculations

6.1.4.1 Total Equipment Sampling

If the total equipment train is sampled, then the SL is simply:

$$\text{Safety Limit (µg/ml)} = \frac{\text{MACO (µg)} \times \text{TRC} \times \text{RF}}{\text{Rinse Volume (ml)}}$$

Where:

Abbreviation	Term	Definition
TRC	Test Result Correction	(Dimensionless) Refer to Section 6.1.4.3
RF	Recovery Factor	(Dimensionless) The fractional amount of residue that can be recovered when sampling

6.1.4.2 Partitioning Sampling

Rinse Sampling

In most cases, rinse samples are taken for individual pieces of equipment. It is not practical to collect and combine all the rinse samples from an equipment train; therefore, the ratio of sample surface area (e.g., surface area of individual piece of equipment) to total equipment train (e.g., sum of all shared equipment surface areas) is included in the SL formula.

The partitioning rinse sampling calculation is performed for each piece of equipment as follows:

$$\text{Separate Equipment Rinse Safety Limit (µg/ml)} = \frac{\text{MACO (µg)} \times \text{SSA (cm}^2\text{)} \times \text{TRC} \times \text{RF}}{\text{Total Equipment Surface Area (cm}^2\text{)} \times \text{Rinse Volume (ml)}}$$

Swab Sampling

Though the most common tool used for direct surface sampling is the swab, the calculations used in this section can be used for any type of partitioning sampling.

The formula is similar to calculating the Separate Equipment Rinse Sampling.

Sample surface area is the area sampled (e.g., 25 cm^2).

[7] Once the cleaning process is qualified, process control limits are set based on a user defined fraction of the calculated MACO value (e.g. action limits and alert levels consistent with the cleaning process performance and capability). Exceeding a process control limit requires an investigation and actions to bring the cleaning process back into control.

Swab SL in mg/swab applies to any swab sample taken on the shared equipment train. It is calculated as:

$$\text{Swab Safety Limit (µg)} = \frac{\text{MACO (µg)} \times \text{SSA (cm}^2\text{)} \times \text{TRC} \times \text{RF}}{\text{Total Equipment Surface Area (cm}^2\text{)}}$$

6.1.4.3 Sample Corrections

The SL may need to be corrected depending on the sampling and testing circumstances.

Sampling Recovery Factors

If there is a requirement to correct for recovery (refer to Section 7.1), this Guide shows the correction applied to the SL instead of the individual test results. If the Recovery Factor (RF) is applied to individual results, the testing laboratory will need to use the correction factor for each sample. When RFs can be grouped for MOC, the method in this Guide is to apply any RF directly to the SL. The SL is multiplied by the % recovery (i.e., in decimal form).

Each company should evaluate the best method for applying RFs.

Test Result Correction

When using a non-specific test method (i.e., TOC, conductivity), the SL is also corrected for signal or interpretation. For example, for TOC the SL is multiplied by the % carbon (i.e., in decimal form) contained in the molecule of interest.

6.1.5 Cleaning Limits for Legacy Cleaning Processes

Legacy cleaning processes may have other types of cleaning limit calculation methods for their cleaning validation program (refer to Section 6.1.1.3); however, a firm needs to adopt the HBEL approach to meet current regulatory expectations.

For existing products, a firm's historically used cleaning limits, if lower than the HBEL-determined residue limit, may be retained as alert levels. An evaluation of data for an existing validated cleaning process should demonstrate that the process's capability provides a sufficient margin of safety. This would show that variations above the HBEL will be prevented.

> *"For existing products, manufacturer's historically used cleaning limits should be retained and can be considered alert limits provided that when taking cleaning process capability into account, they provide sufficient assurance that excursions above the HBEL will be prevented. A similar process should be adopted when establishing cleaning alert levels for products introduced into a facility for the first-time."* [7]

Additional information can be found in the EMA Questions and answers on implementation of risk-based prevention of cross-contamination in production and 'Guideline on setting health-based exposure limits for use in risk identification in the manufacture of different medicinal products in shared facilities' [7].

6.1.6 Acceptance Criteria for Dedicated Equipment

Acceptance criteria for dedicated equipment follow the same principles described in this Guide for multiproduct equipment. The risk for cross-contamination between different products is not present; however there is still need to remove cleaning agents if used, degraded products if present, and other residues based on process understanding.

The FDA Guidance for Industry: Q7 Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients Questions and Answers [20] states:

> *"'Visually clean' may be acceptable for dedicated equipment based on the ability to visually inspect and sufficient supporting data from cleaning studies (e.g., analytical determination to demonstrate cleaning effectiveness)"*

"Regardless of whether equipment is dedicated or not, it is expected that acceptance criteria for residues be defined and that the equipment be cleaned at appropriate intervals to prevent build-up and carry-over of contaminants. Intervals can be based on number of batches, product change-over, time, etc."

"The appropriate interval is confirmed during cleaning validation."

Equipment can be dedicated to a type of material such as non-active materials (e.g., excipients, buffers, medias). If there are no hazards expected from the non-active materials due to degradation, exposure to the environment, or interaction with other components, then a risk assessment can be completed to analyze the cross-contamination risks and support a decision for applying minimum controls. This decision should guide the scope of the validation effort. Remaining residues should not impact product quality or manufacturing processes and the product contact surfaces should be visually clean.

Once the cleaning process is validated, cleanliness effectiveness is monitored at appropriate intervals. (Refer to Section 9.9 for additional guidance on dedicated equipment).

6.2 Acceptance Limits for Fragments of Human Therapeutic Proteins

A rational approach for setting safety-based acceptance limits for fragments of Human Therapeutic Proteins (HTPs), either from API or product manufacturing, is described in this section. This approach is designed to ensure that the carryover of inactive HTP fragments between batches of different products is acceptable from a predictive safety standpoint [67]. See also *ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3].

6.2.1 Inactivation of HTPs during Cleaning and Steaming

Biopharmaceutical cleaning and steaming processes are typically designed to expose product contact equipment to extreme pH (< 2 and > 13) and high temperature (60°C–120°C) for several minutes. Under these conditions, monoclonal antibodies, therapeutic proteins, and other biopharmaceuticals degrade and denature rapidly into pharmacologically inactive fragments [67, 68]. These phenomena can be characterized by exposing the process soil to simulated cleaning and steaming conditions at bench scale [67].

"The bench-scale experiments are designed to simulate full-scale operating conditions that are least conducive (worst-case) for inactivation. The degree of inactivation can be evaluated by subjecting samples and untreated controls to appropriate assays (e.g., sodium dodecyl sulfate polyacrylamide gel electrophoresis [SDS-PAGE] and bioassay can be used to evaluate fragmentation and biological activity, respectively). The results of the study are used to set acceptance limits for the process residue [based on risk to patient safety]. The product inactivation approach is therefore more science-based and reflective of the phenomenological aspects of the cleaning [and steaming] processes." [67]

6.2.2 Safety Profile of Inactive Fragments of HTPs

To address the safety profile question of the inactive fragments, consider

"an equipment train that is used to manufacture Product A. The cleaning and sterilization cycles are known to denature and degrade any residual product in the equipment into fragments that are pharmacologically inactive. The inactive fragments of Product A are carried over into the subsequent batch of Product A. Thus, as a class of molecules, inactive fragments of HTPs do not present a new or unknown risk from a safety standpoint. In fact, these types of fragments have been present in biopharmaceutical products for decades. Further, a comprehensive literature search did not reveal any evidence of safety or efficacy issues attributable to the presence of inactive fragments [of HTPs] in parenteral drugs.[8]

[8] Based on a literature search conducted by R. Sharnez.

To extrapolate the above analysis to multiproduct cleaning validation, consider the introduction of a new product (Product B) into the facility. Part of the equipment train is now used to process both products. The cleaning and sterilization cycles between batches of different products (A → B or B → A, [i.e., intercampaign processing or changeover]) are the same as those between batches of the same product (A → A or B → B, [i.e., intracampaign processing]). Thus, for a given set of cleaning and sterilization cycles, the molecular weight distribution (MWD) of inactive fragments of Product A (IFA) that are carried over into a subsequent batch of Product B (intercampaign processing) is the same as the MWD of IFA that are carried over into a subsequent batch of Product A (intracampaign processing). The same is true for the MWD of inactive fragments of Product B (IFB). Thus, the IFA that are carried over during changeover into Product B do not present a new or unknown risk from a safety standpoint. This implies that the equipment train can be used to manufacture multiple products without introducing a new or unknown class of impurities into any of the products. Further, the carryover of IFA into Product B is significant only for the first lot of Product B that is manufactured following changeover." [67]

6.2.3 Comparable Quality Approach[9]

"The acceptance limits for inactive HTP fragments can be set based on the Comparable Quality (CQ) approach. With the CQ approach, the amount of the target impurity – in this case inactive fragments of Product A – that is carried over into the largest dose (i.e., largest dose that is administered to a patient in a day) of the subsequently manufactured product (Product B) is limited to the acceptable exposure per dose of a reference impurity. The reference impurity must be comparable to or worse than the target impurity from a predictive safety standpoint.

Predictive safety for inactive HTP fragments is evaluated in terms of the key factors that determine toxicity and immunogenicity. For HTPs, toxicity is determined by pharmacological activity; thus, toxicity is generally not a concern for inactive HTP fragments. Immunogenicity is primarily determined by foreignness and chemical complexity.

Chemical complexity increases with molecular weight (MW); thus, larger molecules tend to be more immunogenic. The most active immunogens tend to have a MW greater than 100 kilo Daltons (kDa). HTP fragments with MWs less than 10 kDa are generally weak immunogens. Small polypeptides under 10 kDa usually need to be conjugated to large immunogenic carrier proteins or administered with adjuvants to ensure an antibody response.

The suitability of gelatin as a reference impurity for setting acceptance limits for inactive HTP fragments is evaluated in the next section." [67]

6.2.4 Scientific Rationale for the Use of Gelatin as a Reference Impurity[10]

"The use of gelatin as a reference impurity for inactive HTP fragments is justified for the following reasons:

- *Gelatin consists of a mixture of animal protein fragments derived from the hydrolysis of collagen, a protein that is commonly found in connective tissues. The collagen is hydrolyzed by exposing the connective tissues to pH and temperature extremes. HTPs in the process residue are exposed to similar operating conditions during cleaning and sterilization. Thus, in terms of chemical composition, the protein fragments in gelatin are comparable to the HTP fragments in the process residue.*

- *To elicit an immune response, a molecule must be recognized as nonself by the immune system. The protein fragments in gelatin are of animal origin whereas the HTP fragments in the process residue are of human origin. Thus, the peptide sequences in the HTP fragments are more likely to be recognized by human immune systems than the peptide sequences in the protein fragments in gelatin. Consequently, as compared to the protein fragments in gelatin, the HTP fragments in the process residue are less likely to elicit an immunogenic response in humans.*

[9] Information from Sharnez and To [69], Sharnez et al. [70], Kindt et al. [71], Murphy [72], Hanly et al. [73], as cited in [67].
[10] Information from Sharnez [68], Sharnez et al. [70], Lodish, Berk, and Zipursky [74], Gelatin Handbook [75], as cited in [67].

- *The molecular weights of most of the HTP fragments are typically less than 100 kDa, and a significant fraction of the fragments are less than 10 kDa. HTP fragments with MWs less than 10 kDa are generally weak immunogens.* [In comparison,] *protein fragments in gelatin range from 15 kDa to 400 kDa, which is substantially higher than the molecular weight range of HTP fragments."* [67]

Application of the CQ approach based on gelatin as a reference impurity is described in the next section.

6.2.5 *Application of the CQ Approach to Biopharmaceutical Cleaning Validation*

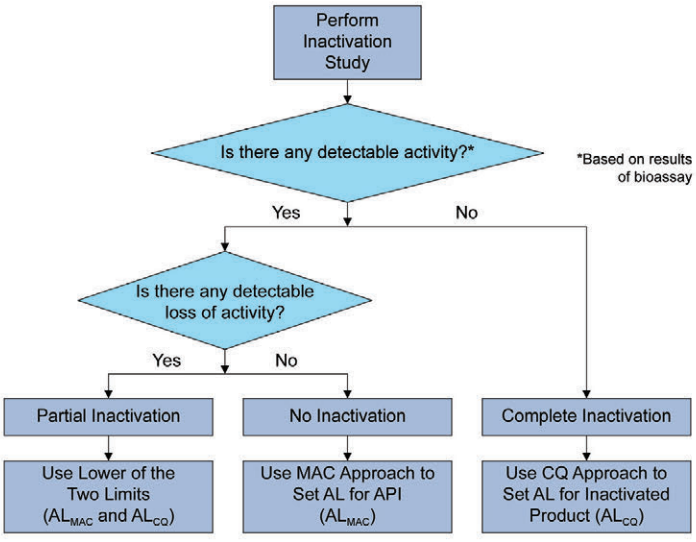
Gelatin is widely used as a stabilizer in many parenteral products. The amount of gelatin in common parenteral products ranges from several hundred micrograms to over 15,000 µg per dose [67].

> *"Lupron [Depot®][11] serves as a good model for the CQ approach because it is administered in multiple doses over an extended period. Note that from the standpoint of immunogenicity, repeated dosing of an immunogen is important; however, dosing frequency is generally not critical provided that the time interval between exposures is relatively short (e.g., weekly, bi-weekly, or monthly)."* [67]

Each dose of Lupron contains 0.65 mg of gelatin; thus, the CQ Criterion based on the gelatin in this product is that the mass of inactive fragments of the previously manufactured product (Product A) carried over into the largest dose of the subsequently manufactured (Product B) must be ≤ 0.65 mg or 650 µg.

> *"The … methodology for setting acceptance limits for multiproduct equipment is summarized in the flowchart.* [See Figure 6.1.] *Inactivation studies are first performed under simulated worst-case operating conditions.*[12] [If the post-cleaning residue (or post-steaming residue, if steaming is performed)*] is shown to have no detectable activity, the CQ approach [based on gelatin is] used to set acceptance limits for the inactivated product (right side of flowchart* [Figure 6.1]*). However, if there is no detectable loss in activity, the conventional MAC approach [based on ADE is] used to set the acceptance limit for the previously manufactured product. If the results indicate that the API is partially inactivated, the acceptance limit is determined for the API as well as the inactivated product, and the lower of the two limits is used. Alternatively, the operating parameters can be modified to ensure inactivation of the API. This can be facilitated by running additional [bench-scale] studies to characterize the effect of the operating parameters on the API." [67]*

Figure 6.1: Flowchart for Setting Acceptance Limits for Inactive Human Therapeutic Proteins
Adapted from JVT [67]



[11] This section is from "Biopharmaceutical Cleaning Validation: Acceptance Limits for Inactivated Product Based on Gelatin as a Reference Impurity" [67]. The product name has been left in for clarity.
[12] Information from Sharnez et al. [76], as cited in [67].

"The acceptance limit for inactive fragments based on gelatin as a reference impurity was ascertained to be 0.65 mg per dose. At 0.65 mg of inactive fragments per dose, the acceptance limit for TOC swab samples was shown to be 3.25 ppm Carbon.[13] This estimate was based on relatively unfavorable [(worst-case)] system parameters. Note that this acceptance limit is substantially higher than the LOQ of TOC, which is typically between 0.05 and 0.2 ppm Carbon. It also compares favorably to the process capability limit (PCL) of most cleaning processes, which is typically on the order of 1 ppm carbon. Thus, with the CQ approach based on gelatin, it is unlikely that the acceptance limit for the process residue would be below the PCL of the cleaning process or the LOQ of TOC."

[The above methodology] *"is not applicable to allergenic ingredients, penicillin, cephalosporin, potent steroids, and cytotoxic compounds.* [Typically, equipment used to manufacture these products are dedicated.] *Acceptance limits for process residues associated with these products are typically set to the limit of detection (LOD) of the best available analytical method."* [67]

6.3 Visual Inspection and Criteria

Visually Clean (VC) is one criterion used to assess surface cleanliness. This criterion is significant in that if there is visible residue on the surface, then the equipment is not considered clean. The visual inspection is an active observation of all visually accessible product contact surfaces of the pharmaceutical manufacturing equipment after every cleaning. It is a GMP requirement [77] and must determine that the equipment is free from any visible residues in order for the cleaning to be considered adequate. Additionally, GMPs require completing a visual inspection immediately before manufacturing activities can commence [78].

6.3.1 Visible Residue Limit Studies

It is possible to conduct Visible Residue Limit (VRL) studies to determine the level of visible detection of residues for many soilants and surfaces. A method to determine the detection level is by spiking decreasing amounts of each residue onto testing coupons representative of equipment surfaces, allowing them to dry, and then having them viewed by a group of observers. Multiple observers should view the residues under different light levels, from different distances, and from different angles to mimic actual visual inspection conditions in order to provide a more rugged visual limit.

"The lowest residue amount that is visible to all observers is the visual detection limit for that product." [3]

If the residues can be consistently observed at a known residue level, and this level is much lower than the cleaning acceptance criteria, then VC provides a high degree of confidence that the equipment is sufficiently cleaned. Even with a visual limit in place, VC is predominately not considered adequate by itself for establishing cleaning validation. Direct surface sampling (e.g., swabs or TOC rinse measures) is also required [17]. However, VC could be used as a criterion along with periodic sampling in a routine monitoring program after the cleaning validation is complete [79]. If historical data shows that the visual limit is higher than what was obtained via coupon studies, launch an investigation to verify. If the out of specification is correct, then raise the VRL.

For the VRL to have value, the data or results for the margin of safety for VI (distance between the safety limit and the residue level represented by the VRL) must be sufficiently large to compensate for the variability between operators performing the VI and also the inherent variability of the VI itself. Operators performing VRL determinations should be qualified in the method. VI for VRL determinations should be performed by an operator and verified by a second operator prior to the equipment being released for use. In addition, a periodic review of the controls is necessary after the cleaning process has been qualified to ensure that performance has not been negatively impacted by increased or new sources of variability and to confirm that a VRL is still a valid and justified approach.

If the VRL is at a level above the cleaning acceptance criteria, then VC has limited value in determining whether the equipment is sufficiently cleaned.

[13] Note that the use of a non-specific method such as TOC also allows for the detection of intact protein.

VRL studies are determined using well-defined parameters to enable its use in cleaning programs and minimize subjectivity. The viewing variables associated with studying visible residue must be defined, and then experimental parameters for the study can be established [80]. The parameters considered are:

- MOC
- Light conditions
- Viewing distance
- Viewing angle
- Observer variability
- Solvent effects

The MOC must match or represent the ones used in the equipment to be cleaned (e.g., SS, polycarbonate, glass, etc.). Conducting VRL studies for all equipment MOCs is not logical. For example, a VRL on a white surface (e.g., PTFE) would be much higher than on a SS surface [81]. Therefore, VRL studies should be coordinated with swab sampling of the equipment to confirm swab results are lower than the VRL. The swab results would also demonstrate equivalent cleaning for all MOCs. After validation, for routine monitoring it could be concluded that if the SS surfaces were visibly clean, then all surfaces were cleaned to the same extent.

Lighting conditions for visual determinations for cleanliness will vary from one piece of equipment to another, and from room to room. Light intensity parameters should be determined for the visual inspection procedure intended to be used. Light intensity levels above 200 lux do not have an impact on visual observations, but light levels below 200 lux inhibit the ability to detect visible residues [80].

The viewing distance and viewing angle are based on the manufacturing equipment that is used at the site. Larger pieces of equipment can often be viewed at a distance of no greater than 10 feet and could have a restricted viewing angle [80].

The variations in observers can be minimized by implementing clear procedures, training observers on how to conduct visual inspections consistently, and establishing the VRL parameters as controls for VI determinations.

A recommended training approach for inspectors [78] includes:

- Reviewing SOP for conducting the visual inspection of cleaned manufacturing equipment, including product contact surfaces
- Reviewing equipment diagrams to understand hard-to-clean areas and areas of product buildup
- Reviewing VRL examples
- Discussing VRL versus cleaning limit
- Conducting on-the-job training
    - Emphasize effects of viewing parameters, especially viewing angle
    - Harmonize on when to use supplemental lighting (flashlight/torch)
    - Strive for consistency among visual inspections to maintain expectations for visual cleanliness

Visual inspections are normally executed by qualified personnel and documented in cleaning log books or manufacturing batch records.

Refer to Appendix 3 – Example: Protocol for Development and Establishment of a Visible Residue Limit (VRL).

6.3.2 Visible Residue Limit and Safety Limits

It can be justified and documented that for chemicals and actives where the VRL is significantly (e.g., lower than 20% of the margin of safety) lower than the SL, equipment surfaces cleaned to a VC level following cleaning of the previous product would ensure with low risk the next manufactured product's identity, strength, quality, and purity from a clean equipment perspective.

> *"Assuring adequate removal of non-intrinsic cleaning agents such as surfactants often involves very high health-based residual limits and the absence of visible residue is a much more stringent criteria than the health-based residual limit."* [3]

6.3.3 Non-Accessible Areas and the Visual Inspection Process

Product quality concerns should be addressed for areas not accessible to visual inspection. Some methods include a cleaning comparison evaluation or boroscoping. A risk-based approach should be applied when using either of these methods, especially for actives with low ADE/PDE (high hazard) values.

6.3.3.1 Cleaning Comparison Evaluation

If equipment that is not visually accessible (e.g., a transfer line) is cleaned with a cleaning process as robust, or more robust than equipment that is visually accessible, it may be argued that the visually inaccessibly equipment is also clean.

The cleaning of the inaccessible areas needs to be equal to or greater than the cleaning of accessible areas. For example, a tank and transfer line with the same production soil are cleaned using the same cleaning parameters (i.e., cleaning time, cleaning agent concentration, and temperature). The only difference is the cleaning action. The tank is cleaned using impingement and cascading action, and the transfer pipe is cleaned using turbulent flow. If the piping cleaning flow meets the appropriate turbulent flow, it can be rationalized that the transfer line is clean to a visual cleanliness level since the tank areas cleaned via cascading action were demonstrated to be VC.

This rationale could also be used for parts of the same equipment. For example, if a spool piece (i.e., determined to be VC after cleaning) on the transfer line system was cleaned via the same cleaning process and shown to be as difficult or more difficult to clean as the rest of the transfer line system, it could represent the whole transfer line system.

It is not necessary to demonstrate that all visually accessible area cleaning actions (i.e., impingement and cascading in the case of the tank) are less than or equal to the visually inaccessible area cleaning action to make the argument. Each situation should be evaluated individually while considering the cleaning process and cleaning difficulty.

6.3.3.2 Boroscoping

Pipes, transfer lines, and other inaccessible areas can be visually inspected using a boroscope. Any remote visual inspection should be qualified. The benefit of boroscoping inaccessible areas should be weighed against the equipment maintenance and longevity risks of routinely breaking line connections and performing intrusive inspections. The decision for or against routine boroscoping for visual inspection should be thoroughly risk assessed.

6.3.4 Organoleptic Inspection

The removal of odors or color residues may need to be confirmed as part of cleaning validation. Where the requirement is not based on safety, and where there are no calculated residue limits, the acceptable removal of the residue may be performed by using the sense of smell or through visual inspection.

Visual inspection of the surfaces can be quantified and so may also be used were a residue limit is calculated.

Detection of odors can be enhanced by sealing the equipment item overnight. For example, by closing a lidded vessel or containing the equipment in a bag, any odor is concentrated, giving a greater assurance that no odor will be carried over into the next product.

Those involved in manufacturing may become desensitized to the odor; therefore, the test is more effective if it is performed by someone not involved in the manufacture of that product.

The level of qualification should be risk based. The level of the risk (i.e., business risk or product risk) should match the level of qualification.

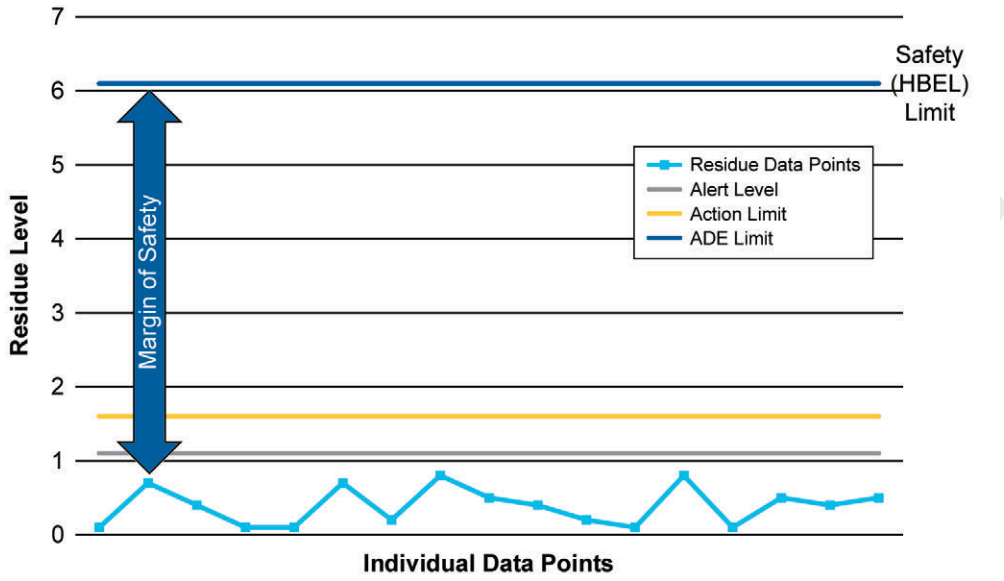
The detection of colored products may be enhanced by examining a swab used to sample difficult-to-inspect points where material may gather, for example seal interfaces. However, detection of residual color during sampling is already too late to prevent a cleaning failure. A more practical approach for a piece of equipment having a color or dye ingredient is to thoroughly wipe the entire surface of the equipment with solvent (e.g., 70% isopropyl alcohol) as the last step of the cleaning process. This is often done during cleaning to remove residual water, but can also serve as a check on color removal before the equipment gets to the visual inspection and sampling steps.

6.4 Process Consistency, Capability, and Control

A validated process cleans surfaces consistently to a certain residue level. This level takes into consideration all sources of variability and represents a measure of process capability. A process that is in control will meet its cleaning residue limits consistently. A statement of process consistency is made when the cleaning process is qualified for the first time. Additional data is collected during the process monitoring phase to establish appropriate limits for process control purposes.

The difference between the residue levels obtained by executing the cleaning process and the safety-based acceptance limit represents the margin of safety of the cleaning process (see Figure 6.2).

Figure 6.2: Example of a Process Control Chart for a Cleaning Process when HBEL is Used



Process Capability (C_p) is defined as a statistical measure of the inherent process variability of a given process characteristic and involves calculating the ratio of the specification limits to process spread. For cleaning validation, the C_p can be adapted as an index to measure cleaning process performance against the cleaning validation residue limit (e.g., the SL or a lower cleaning limit defined for the process), which is considered the upper side of the specification. This is called the Upper Process Capability Index, or C_{pu}. Therefore, to evaluate the cleaning process C_{pu}, gather the residue data of the cleaning process (data sample of at least 25 values from cleaning development work and/or cleaning qualification runs), calculate an average and a standard deviation for the residue data sample, and calculate a C_{pu} index with the following formula:

$$C_{pu} = \frac{[(\text{Cleaning Limit}) - \text{Average of data sample}]}{(3*\text{SDEV of data sample})}$$

Where: $C_{pu} = C_{pk}$ index using the cleaning limit as the upper specification of the cleaning process
Average = Statistical average of the residue data sample
SDEV = Standard Deviation of the residue data sample

In order for the C_{pu} index to provide useful information, the cleaning process should be in control, stable, the residue data should represent a normal distribution of data (or normalized using statistical principles), and the residue data should be a representative sample of the process.[14]

The resulting index provides a statistical assessment of how well the cleaning process is performing against the cleaning limit. An index at 1.0 represents a capable process, and an index above 1.3 indicates a robust process capability. The larger the cleaning C_{pu}, the greater the probability that the residues will not exceed the SL, providing a large margin of safety and ensuring a cleaning process that remains within statistical process control [82].

After a sufficient number of data points (i.e., cleaning validation data and routine monitoring sample results) have been collected, a program is implemented to continuously monitor and trend the performance and effectiveness of cleaning. Statistical Process Control (SPC) tools can be used to monitor and control the process. Typically, target residue limits (alert levels, action limits) are defined to assess continued control of the cleaning process. If consecutive data points (i.e., trend) exceed alert levels, actions are taken to mitigate further process drift. If a data point exceeds the action limit, the cleaning process is considered out of control and a root-cause investigation is performed to identify the reason for the failure. Corrective actions are implemented to bring the cleaning process back to a controlled state and to avoid recurrence of the failure.

The variability of a manual cleaning process is different from an automated process (e.g., CIP). Due to this higher variability, the control limits for a manual process could be wider than for a CIP process.

Rational Approach to Establish a Control Strategy for New Cleaning Processes using HBEL-Based Cleaning Safety Limits, Alert Levels, and Action Limits

Once the SL based on HBEL is determined, the process design information is used via a QRM process (refer to Chapter 3) to determine if sufficient controls (organizational, technical) are in place to ensure an effective and stable cleaning process. Although residue limits should be based on HBELs, it is not intended to be used to set routine cleaning limits at the level of the calculated HBEL. The objective is to establish the cleaning process in such a way that it operates with sufficient controls to prevent residues that exceed the SL. This can be achieved by establishing the process control cleaning limit based on the process variability and capability.

The following steps describe a rational approach for process control:

1. Determine a SL based on HBELs.

2. Review the cleaning process information and apply QRM principles to confirm that sufficient controls are in place for a stable and effective cleaning process.

[14] Additional statistical adjustments may be necessary to obtain an estimate of the capability index when less than 25 sample points are available.

3. Execute cleaning validation process PQ runs with the predefined acceptance criteria (SL, visual inspection, number of qualification runs for consistency, bioburden controls).

4. Assess the cleaning process margin of safety by calculating a process capability index against the SL (C_{pu}) per the C_{pu} formula. A process capability index greater than 1.3 represents a robust process with a high probability of not exceeding the SL.

$$C_{pu} = \frac{[(\text{Cleaning Limit}) - \text{Average of data sample}]}{(3*\text{SDEV of data sample})}$$

Where: $C_{pu} = C_{pk}$ index using the cleaning limit as the upper specification of the cleaning process
Average = Statistical average of the residue data sample
SDEV = Standard Deviation of the residue data sample

5. Once the cleaning process is qualified, establish process control parameters:

   a. Compare SL against a data set consisting of actual residue levels obtained from the cleaning process. All relevant data should be considered, including data generated during cleaning process development and/or data from the cleaning process qualification.

   b. Evaluate the variability of this data set by calculating a sample average and a sample standard deviation.

   c. Establish cleaning process action limits as the initial control limits using statistical principles and tools (e.g., average + 3*SDEV of the residue data).

   d. Establish cleaning process alert levels using statistical principles and tools.

6. Monitor the cleaning process. Investigate trends in alert levels and excursions above action limits as necessary to ensure the process remains in control.

7. Periodically assess if the alert levels and/or action limits are still appropriate to control the cleaning process.

6.5 Bioburden and Endotoxins

Bioburden and endotoxin controls prevent the proliferation of microbial loads and endotoxin limits on process equipment product contact surfaces. Measurement of bioburden limits is necessary to establish CHT, and in some operations, a bioburden and endotoxin limits are established as a criterion for cleaning validation. Acceptable levels of bioburden and endotoxin are assessed through a risk assessment. Once acceptable levels of bioburden and endotoxin are justified, they are challenged and documented in the cleaning validation. A risk assessment should determine if routine bioburden and endotoxin monitoring is necessary post validation.

6.5.1 Microbial Acceptance Criteria Calculation

After the recovery studies discussed in Appendix 4 and Appendix 5 have been developed and validated, it is acceptable to use them for cleaning validation and routine monitoring. The formulas in Section 6.5.2 can apply to any equipment surface for non-sterile DPs.

To date there are no regulatory requirements for microbial limits or recovery for cleaning validation for non-sterile or sterile products. The regulatory expectation to manufacture GMP products is control of bioburden [83]. However, there are many different methods that can be applied to establish bioburden and endotoxins limits. For example, bioburden and endotoxin in the next product can come from many additional sources like environment, raw materials, people, etc. These sources should be considered when developing acceptance criteria. The recovery of 50% or greater for most microorganisms is based on recovery tests performed on various strains. Most companies have an internal requirement that specifies any value below 50% RF requires a correction factor to be applied to the recovery value.

Consider that microbial contamination found by sampling in the equipment is only loosely related to the actual contamination, because (as opposed to chemical contamination) microorganisms may die, be inhibited, stay in latent life, or multiply (grow). This is why the requirement for recovery in microbiological cleaning validation is not as strict as in chemical cleaning validation, and also why microbiological acceptance criteria are based more on historical values and trending rather than on fixed figures.

Typically, a % Recovery Correction Factor (RCF) is applied because of low bioburden recovery from the swab and/or contact plate recovery studies. The RCF is applied by the QC microbiology laboratory after post-cleaning bioburden results are reviewed.

6.5.2 Non-Sterile Surface Bioburden Limits Calculations

According to current USP <1111> Microbiological Attributes of Nonsterile Pharmaceutical Products [46], the significance of microorganisms in non-sterile pharmaceutical products should be evaluated in terms of the use of the product, the nature of the product, and the potential hazard to the user. For non-sterile products, endotoxin sampling of equipment product contact surfaces is not necessary.

Also, the FDA GMP requirements specified in 21 CFR Part 211.113, Control of Microbiological Contamination, [83] state:

> *"Appropriate written procedures, designed to prevent objectionable microorganisms in drug products not required to be sterile, shall be established and followed."*

When developing acceptance criteria for non-sterile bioburden limits post cleaning, it is important to consider the surrounding environment to which the equipment product contact surfaces are exposed. The microbiological quality of air classification of the area should be considered for bioburden limits for equipment product contact surfaces. For example, if product is manufactured in a cleanroom Grade D [84] or ISO 8 [85], then these bioburden limits should be considered. However, the product contact surfaces should never exceed bioburden limits for area in which the equipment is exposed. Regardless of the method used, it is still important to determine the risk and impact of bioburden limits on product stability, safety, and efficacy.

The calculation of the accepted microbiological limits in the equipment is similar to chemical cleaning validation. With the specification of the accepted contamination[15] and the smallest batch size in the equipment train (in **weight**), the overall microbial contamination in that train can be calculated.

The resulting calculation (below) is based on post-cleaning limits, and the % recovery used in the calculations below is arbitrary as an example in the formula. In some cases, for non-sterile product, a safety factor is applied because equipment is not sterilized post cleaning. Therefore, the risk is higher for bioburden levels to increase during an extended CHT.

It is important when developing bioburden acceptance criteria to consider other process variables such as equipment wetted versus dried surfaces, DHTs and CHTs, as well as raw and in-process material bioburden load.

Note: Extended CHTs and DHTs require validation. For companies that do not want to validate their CHTs, when equipment is not used within 24 h, recleaning is required.

6.5.2.1 Applying Bioburden Recovery Correction Factor (RCF) (Optional Approach)

As described in Section 8.2, the limitation of bioburden recovery may need to be considered in the samples listed below. For instance, using the examples in Appendix 4, if a coupon is inoculated (spiked) with 100 CFU/25 cm^2 (for each of the five challenge test organisms) and the average recovery rate of the five test organisms is less than 50% (50 CFU), then theoretically there still is 50% (50 CFU) of the spiked microorganisms remaining on the coupon surface.

[15] For nonaqueous oral use, 10^3 CFU/g aerobic bacteria and 10^2 CFU/g molds and yeasts and for aqueous oral use, 10^2 CFU/g aerobic bacteria and 10^1 CFU/g molds and yeasts [46].

RCF should only be considered if all conditions for test method development and validation were followed and bioburden results were still low. If an RCF is used, it should be based on the average of the five challenge microorganisms, the main reason being that it is difficult to apply five different RCFs to post-cleaning microbial test results. The microbiology QC laboratory may consider applying an RCF to the CFU data obtained during cleaning validation studies to compensate for the limitation in the test method assay, but only if the conditions listed in the "Considerations before Applying an RCF" are met.

6.5.2.2 Considerations Before Applying an RCF

Direct Method (Swabs and Contact Plates)

Applying an RCF should only be used if recovery results are low (less than 50% for the average of five challenge microorganisms) and a detailed investigation concludes that the following items were performed and deemed acceptable:

- The swab or contact method (direct method) was effective
- The recovery studies have shown no errors caused by the inoculation solution or method
- There were no laboratory technician sampling methods or extraction method errors
- Test coupons were properly cleaned and sterilized
- It was verified that there was no chemical residue on the coupon that could interfere with the recovery study test results and method
- A recovery solution (inoculation) such as Phosphate-Buffered Saline with 0.04% Tween 80® (PBST) or other effective solution was used to spread, stabilize, and prevent desiccation (drying) of inoculated (spiked) microorganisms on coupons
- Growth promotion results of sampling media were acceptable

Before an RCF is applied, all microbiological assay variability must be investigated, identified, and corrected.

Indirect Method (Rinse Sample) Considerations

Low recovery of microorganisms in the final rinse (indirect method) water sample after cleaning may not necessarily mean there is low bioburden. It could signal that either the residue cleaning agent or the chemical properties of the product have inhibited microbiological growth. When performing bioburden recovery studies on final rinse samples, it is critical to consider if small amounts of residue chemicals (cleaning agent or product) will impact the test results. Therefore, it is important to perform bacteriostasis and fungistasis studies on the final rinse samples to determine if there are any antimicrobial or inhibition properties.

6.5.2.3 Establish Bioburden Limits for an Oral Solid Dosage Form

The following are some industry-related methods for calculating bioburden on non-sterile surfaces. Typically, the industry approach is to use 25 or 50 CFU per 25 cm^2; however, this is not based on a scientific approach. The formula used below can be considered more scientific when compared to arbitrary values. Even though 25 or 50 CFU per 25 cm^2 is based on arbitrary values, it allows for some degree of bioburden control. It is recommended that companies compare the formula below against the industry standard and choose the lower value. The calculated formula below can also be used as part of a justification for product release during a non-conformance event because from a risk perspective, it demonstrates patient and product safety.

Calculation and Limits for Nonaqueous Products (Non-Sterile)

Microbial Limit for an oral solid dosage form, i.e., tablet or capsule [46]
Total Aerobic Microbial Count NMT 1000 CFU/g
Total Combined Yeast and Mold Count NMT 100 CFU/g
Absence of USP indicator organisms, i.e., *E. coli, S. aureus, and Salmonella spp.*

Approach: The microbial limit in terms of CFUs per 25 cm^2 can be determined from a knowledge of the internal surface area of the equipment train, the quantity of granulation processed, and the microbial limit for the nonaqueous product (oral solid dosage form, also applies to powder)

Example: An equipment train has an internal largest surface area of 1,114,000 cm^2, and smallest batch 130 kg of granulation.

Formula:

Internal Surface Area = 1,114,000 cm^2
Batch Size = 130 kg or 130,000 g
Allow Limit =1000 CFU/g
Swab Surface Area = 25 cm^2
Limit: Aerobic Count NMT = 1000 CFU/g [46]
Limit: Yeasts and Molds Count NMT = 100 CFU/g [46]
Safety Factor of 0.01 Non-Sterile Product (risk factor applied for process equipment that is not sterilized after cleaning)

Given that the batch size is 130,000 g and there can be no more than 1000 CFU/g, the total number of CFU is:

130,000 g × 1000 CFU/g = 130,000,000 CFU

The number of CFU per cm^2 equals the total number of CFU divided by the number of cm^2:

130,000,000 CFU ÷ 1,114,000 cm^2 = 116.7 CFU/cm^2

If you want to include a safety factor of 0.01 (if required based on non-sterile product):

0.01 × 116.7 CFU/cm^2 = 1.167 CFU/cm^2

If the sample size is 25 cm^2:

1.167 CFU/cm^2 × 25 = 29.17 CFU/25 cm^2 ≈ 29.2 CFU/25 cm^2

Acceptance Criteria based on CFU/25 cm^2 is:

29.2 CFU/25 cm^2 (Aerobic) or 3.0 CFU/25 cm^2 (Yeast and Molds)

Calculation and Limits for Aqueous Products (Non-Sterile)

Microbial Limit for an oral liquid dosage form, i.e., liquids [46]
Total Aerobic Microbial Count NMT 100 CFU/ml
Total Combined Yeast and Mold Count NMT 10 CFU/g
Absence of USP indicator organisms, i.e., *E. coli, S. aureus and Salmonella spp.*

Approach: The microbial limit in terms of CFUs per 25 cm^2 can be determined from a knowledge of the internal surface area of the equipment train, the quantity of liquid processed, and the microbial limit for the nonaqueous product (oral liquid dosage form).

Example: An equipment train has an internal surface area of 50,850 cm^2, and a capacity of 500 L of oral liquid dosage form.

Formula:

Internal Surface Area = 50,850 cm^2
Batch Size = 500 L or 500,000 ml
Swab Surface Area = 25 cm^2
Limit: Aerobic NMT 100 CFU/ml [46]
Limit: Yeats and Molds NMT 10 CFU/ml [46]
Safety Factor of 0.01 Non-Sterile Product (risk factor applied for process equipment that is not sterilized after cleaning)

Given that the batch size is 500,000 ml and there can be no more than 100 CFU/ml, the total number of CFU is:

500,000 ml × 100 CFU/ml = 50,000,000 CFU

The number of CFU per cm^2 equals the total number of CFU divided by the number of cm^2:

50,000,000 CFU ÷ 50,850 cm^2 = 983.3 CFU/cm^2

To include a safety factor of 0.01:

0.01 × 983.3 CFU/cm^2 = 9.833 CFU/cm^2

If the sample size is 25 cm^2:

9.833 CFU/cm^2 × 25 = 245.8 CFU/25 cm^2

Acceptance Criteria based on CFU/25 cm^2 sample area is:

245.8 CFU/25 cm^2 (Aerobic) or 24.6 CFU/25 cm^2 (Yeast and Molds)

6.5.3 Sterile Surface Bioburden Limits Calculations

For biological or biotechnology products there should be consideration for two sets of limits. Bioburden limits for upstream processes are typically less stringent than downstream. The closer you get to the final product, the more stringent the limits become. Most companies utilize historical data to establish upstream bioburden, and if no historical data exists, initially the USP WFI specification [46] (10 CFU/100 ml) is used until limits can be established on site.

When using PW or WFI specifications for establishing bioburden limits, it is important to understand that when measuring absolute concentration within a volume, microbial contribution from the surface area should also be considered. This is important because the addition of more rinse water to the cleaning rinse cycle dilutes the amount of CFU/ml detected from the surface. Most regulatory agencies consider this activity to be intentionally rinsing the surface into compliance. The greater the volume of water used, the greater the negative impact on the analyst's ability to discern contamination from the surface. Therefore, it is important to consider rinse volumes and surface areas rinsed as a controlled ratio.

For sterile products, most bioburden limits for post-cleaning final rinse are based either on the specification for USP PW (100 CFU/ml) or WFI (10 CFU/100 ml) [46]. (These specifications are typically used for downstream processes.) This approach can also be applied to the endotoxins specification.

There are no regulatory requirements for bioburden or endotoxins limits for pre-cleaning. However, it is expected that the cleaning process show a minimum of 2–3 log reductions (sanitization levels) from pre-cleaning to post cleaning. For process vessels or systems that require SIP, the bioburden levels should be at least 2–3 logs (10^2–10^3) lower than the sterilization challenge microorganism *Geobacillus stearothermophilus* 6 logs (10^6).

The concerns with having high levels of bioburden post cleaning is that it may surpass or come close to the validated sterilization challenge microorganism *Geobacillus stearothermophilus* 6 logs. In addition, if the equipment is held dirty for an extended period of time the bioburden levels may increase to a point where cleaning the surfaces may be difficult. Therefore, it is recommended to implement a safety factor for bioburden levels post cleaning of minimally 2 to 3 logs lower than the validated sterilization cycle.

In some cases, for sterile products a safety factor is not applied for bioburden because the equipment is typically SIP sterilized post clean.

The formula and acceptance criteria in Table 6.4 can apply to any equipment surface for sterile DPs.

Table 6.3: Acceptance Criteria for Sterile Products

Critical Process Parameters (CPPs)	Process Step	Sample Method	Acceptance Limits (CQAs)
Dirty Hold Time (DHT)	Standard for process tanks < 24 h Mandatory cleaning after 24 h	Swab or Contact plates	To be determined
Initial Purified Water (PW) Rinse Cycle	Ambient temperature (lower temperature will not bake protein onto surface of tank) Samples are taken within first few minutes of start of initial cycle and a few minutes before the end of initial rinse cycle	Rinse Sample	First and Second Rinse Samples: To be determined (Should show some decrease in bioburden from the initial rinse (at the beginning of rinse cycle) to the end of the final rinse cycle. Typically, 2 to 3 log reduction in bioburden.)
Final Water for Injection (WFI) Rinse	WFI at 70°C for a specific time period	Rinse Sample	Action: WFI > 10 CFU/100 ml Alert: WFI ≥ 5 CFU/100 ml Endotoxin: 0.25 EU/ml Calculate total surface area, rinse volume, sample volume = total CFU/per surface area
After Cleaning Cycle	After the final rinse cycle and the drying cycle	Swab or Contact plates	Based on equipment surface area calculation

6.5.3.1 Calculation and Limits for Aqueous Products (Sterile)

The following are some industry methods used for calculating bioburden on post-cleaning surfaces. This calculation assumes that the worst-case microorganism is spread evenly across all surface areas.

Applying the Direct Surface Sampling Method

Microbial Limit for a Biotechnology Process, i.e., Liquid Media
Total Aerobic Microbial Count NMT 10 CFU/100 ml (Based on WFI Specification [46])

Approach: The microbial limit in terms of CFUs per 25 cm^2 can be determined from a knowledge of the internal surface area of the equipment train, the quantity of liquid medium or product (protein) processed, and the microbial limit for WFI specifications.

Example: An equipment train has an internal surface area of 2,000,000 cm^2, and a capacity of 16,000 kg of liquid product (biotechnology growth media).

Formula:

Internal Surface Area = 2,000,000 cm^2
Smallest Batch Size = 16,000 kg or 16,000,000 ml
Swab Surface Area = 25 cm^2
WFI Specifications Limit: NMT 10 CFU/100 ml or 0.1 CFU/ml
No Safety Factor – Equipment SIP after cleaning

Given that the batch size is 16,000 kg or 16,000,000 ml and there can be no more than 0.1 CFU/ml, the total number of CFUs is:

10 CFU/100 ml = 0.1 CFU/1 ml

16,000,000 ml × 0.1 CFU/ml = 1,600,000 CFU

The number of CFU per cm^2 equals the total number of CFU divided by the number internal surface area in cm^2:

1,600,000 CFU ÷ 2,000,000 cm^2 = 0.8000 CFU/cm^2

If the sample area is 25 cm^2:

0.8000 CFU/cm^2 × 25 cm^2 = 20.00 CFU/25 cm^2 ≈ 20 CFU/25 cm^2

6.5.3.2 Calculate Bacteria Limits for Final Rinse

In order to calculate the final rinse water acceptance criteria for endotoxin, it is necessary to know the volume of the final rinse water. For the purpose of this calculation we will assume a final rinse water volume of 150 L. The formula below is an indirect sample method and the dilution ratio is large, therefore a safety factor is not required. The following are some industry-related methods for calculating bacteria on post-cleaning surfaces.

This calculation assumes:

- The worst-case bacteria is spread evenly across all surface areas
- Microbial Limit for a Biotechnology Process, i.e., Liquid Media
- Total Bacteria Count 0.1 CFU/ml (based on WFI Specification [46])

Approach: The bacterial limit in terms of CFU per 25 cm^2 can be determined from a knowledge of the internal surface area of the equipment train, the quantity of liquid medium or product (protein) processed, and the bacteria for utilizing WFI specifications [46].

Example: An equipment train has a capacity of 250 L of liquid product (biotechnology growth media).

Formula:

Final Rinse Volume: 150 L = 150,000 ml
Limit: NMT 10 CFU/100 ml or 0.1 CFU/ml [46]
Smallest Batch Size = 250 L or 250,000 ml

The acceptance limit is calculated by multiplying the smallest batch size (ml) by the allowable limit (CFU/ml, USP WFI Specification [46]), and then dividing by the final rinse volume (ml):

250,000 ml × 0.1 CFU/ml = 25,000 CFU

25,000 CFU ÷ 150,000 ml = 0.1667 CFU/ml ≈ 0.2 CFU/ml in final rinse water

6.5.4 Endotoxin Limits from Surface Sampling

Endotoxin levels can also be established from rinse and swabs samples. Interference from cleaning agents and products should be determined when employing any test method. Alert levels and/or action limits should be established for both methods.

The FDA Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers [86] contains the following information:

"The current good manufacturing practice (CGMP) regulations for finished pharmaceuticals and the medical device quality system regulations require development of controls that include scientifically sound and appropriate sampling plans."

"Sampling plan information is addressed in AAMI ST72, but not USP Chapter <85>. Firms should include a sampling plan as part of their application documentation. In the sampling plan, firms should consider the potential for contamination in raw materials, in-process materials, and the finished product. Specifically, firms should take into account aspects of the manufacturing design, including consistency of a manufacturing process, impact of in-process hold times, endotoxins removal steps, and finished product endotoxins specifications. The sampling plan should be considered dynamic; firms should begin with maximum coverage and adjust their sampling plans as they gain confidence in the prevention of endotoxins in their manufacturing processes. Firms should update their regulatory filings when adjusting sampling plans."

Agents such as EDTA and heparin are known to affect the assay if present in sufficient concentrations. Also, certain cleaning agents could give false positive results; therefore, it is extremely important to perform enhancement studies beforehand to prevent incorrect results. All assays, independent of methodology, are standardized using endotoxin in water. Therefore, unless the sample is water, some components of the solution may interfere with the Limulus Amebocyte Lysate (LAL) test such that the recovery of endotoxin is affected.

If the product being tested causes less endotoxin recovery than expected, the product is inhibitory to the LAL test. Products that cause higher than expected values are enhancing. Overcoming the inhibition and enhancement properties of a product is required by the FDA as part of the validation of the LAL test for use in the final release testing of injectables and medical devices [86].

Endotoxin recovery from materials such as polypropylene can be difficult to achieve because the outer layer of Gram negative bacteria consists of surface proteins, lipoproteins, and phospholipids surrounding Lipopolysaccharide (LPS) molecules. LPS will bind to the polypropylene making it difficult to detect and recover. When spiking recovery studies for plastics and polypropylene materials typically found in carboys, etc., a specific extraction solution will need to be developed to remove the endotoxins from the coupon surfaces.

6.5.4.1 Calculate Endotoxin Limits for Final Rinse

In order to calculate the final rinse water acceptance criteria for endotoxin, it is necessary to know the volume of the final rinse water. In the case of the formula below this an indirect sample method and the dilution ratio is large, therefore a safety factor is not required. The following is an industry-related method for calculating endotoxin on post-cleaning surfaces.

This calculation assumes:

- The worst-case endotoxin is spread evenly across all surface areas
- Microbial Limit for a Biotechnology Process, i.e., Liquid Media
- Total Endotoxin Counts 0.25 EU/ml (based on WFI Specification [46])

Approach: The endotoxin limit in terms of EUs per 25 cm^2 can be determined from a knowledge of the internal surface area of the equipment train, the quantity of liquid medium or product (protein) processed, and the endotoxin for the utilizing WFI specifications [46].

Example: An equipment train has an internal surface area of 50,850 cm^2, and a capacity of 250 L of liquid product (Biotechnology growth media).

Formula:

Final Rinse Volume: 150 L = 150,000 ml
Limit: NMT 0.25 EU/ml [46]
Smallest Batch Size = 250 L or 250,000 ml

Smallest Batch Size (ml) multiply allowable EU/ml (USP WFI Specification [46]) divided by final rinse volume ml = acceptance limits

250,000 ml × 0.25 EU/ml = 62,500 EU

62,500 EU ÷ 150,000 ml = 0.4167 EU/ml ≈ 0.4 EU/ml in final rinse water

6.5.4.2 Calculate Endotoxin Limits for Surface Sample

This calculation assumes:

- The worst-case endotoxin is spread evenly across all surface areas
- Microbial Limit for a Biotechnology Process, i.e., Liquid Media
- Total Endotoxin Counts 0.25 EU/ml (based on USP WFI Specification [46])

Since SIP sterilization will not decrease endotoxin levels a safety factor should be applied to post-cleaning calculations for direct sample method.

Approach: The endotoxin limit in terms of EUs per 25 cm^2 can be determined from a knowledge of the internal surface area of the equipment train, the quantity of liquid medium or product (protein) processed, and the endotoxin for the utilizing WFI specifications [46]. This is direct surface sampling method.

Example: An equipment train has an internal surface area of 50,850 cm^2, and a capacity of smallest batch size 250 L of liquid product (Biotechnology growth media).

Formula:

Internal Surface Area = 50,850 cm^2
Smallest Batch Size = 250 L or 250,000 ml
Swab Surface Area = 25 cm^2
Limit: NMT 0.25 EU/ml [46]
Safety Factor of 0.01

Given that the batch size is 250 L (250,000 ml) and there can be no more than 0.25 EU/ml therefore the total number of EUs is:

250,000 ml × 0.25 EU/ml = 62,500 EU

The number of EUs/cm^2 equals the total number of EUs divided by the surface area cm^2

62,500 EU ÷ 50,850 cm^2 = 1.229 EU/cm^2

To include a safety factor of 0.01:

0.01 × 1.229 EU/cm^2 = 0.0123 EU/cm^2

For a sample area of 25 cm^2, the endotoxin limit is:

0.123 EU/cm^2 × 25 cm^2 = 0.3073 EU/25 cm^2 ≈ 0.3 EU/25 cm^2

6.6 Summary of Acceptance Criteria Approaches for Cleaning Process Performance Qualifications

Approaches for acceptance criteria have been presented in this chapter and are summarized in Figure 6.3 and Table 6.4. Figure 6.3 depicts a flow diagram describing a general approach for selecting acceptance criteria, which includes bioburden and endotoxin controls, visual inspection criteria, residue limits, and process consistency. Once the process is qualified, ongoing monitoring and appropriate control limits are established to ensure process control.

Table 6.4 summarizes the recommended approaches for these criteria, based on the type of active ingredients or chemicals to clean and the type of technology used in manufacturing (e.g., sterile manufacturing). As noted, the use of alternate criteria or approaches could be considered acceptable by regulatory agencies if scientifically justified and agreed to by both parties.

Figure 6.3: General Approach for Cleaning Process Performance Qualification Acceptance Criteria

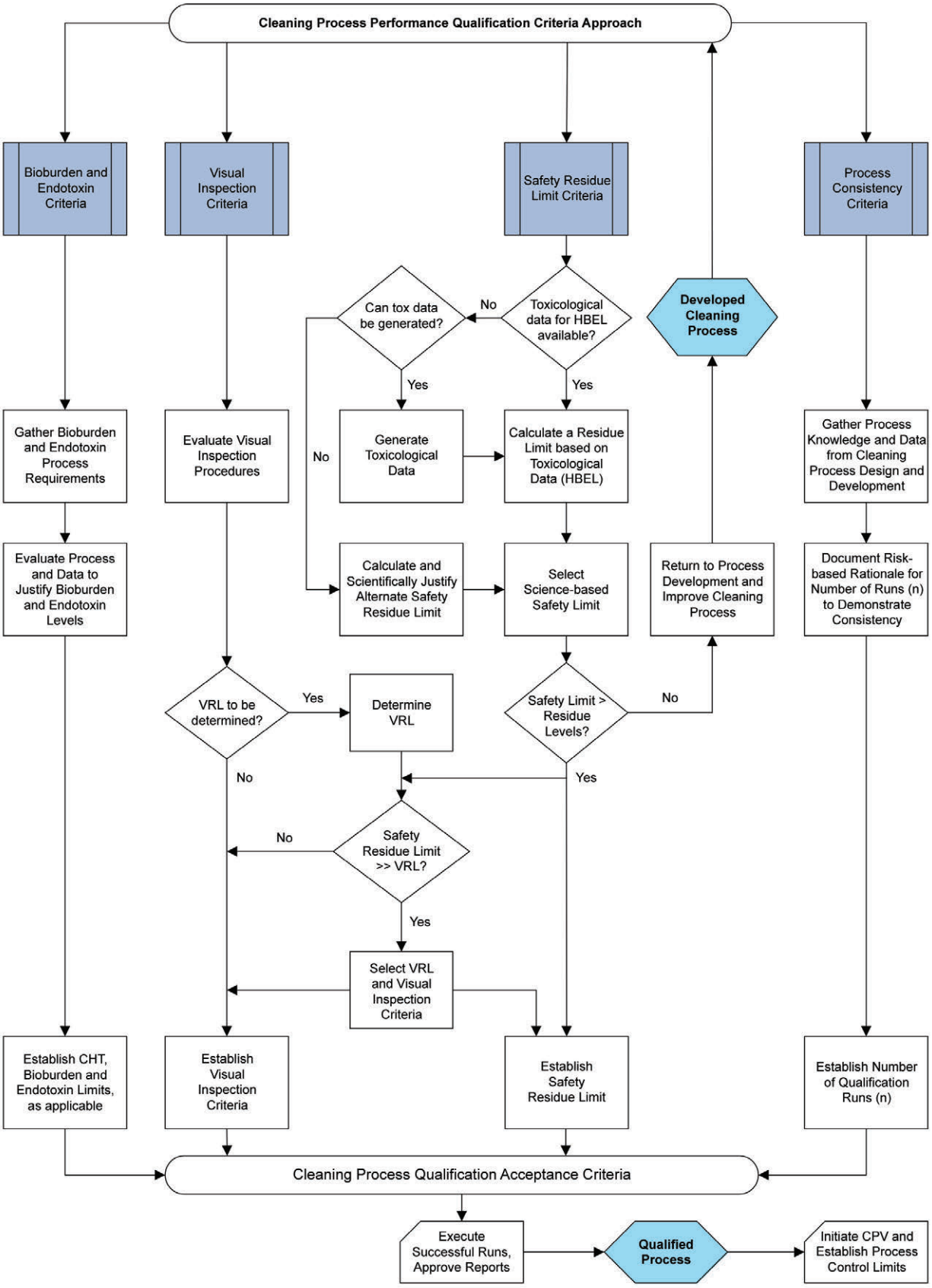


Table 6.4: Approaches[1] for Cleaning Validation Acceptance Criteria for Shared Equipment

Type of Residue to Clean[2]	Safety Limit			Other Criteria			
	HBEL	TTC	R&D	Bioburden	Endotoxin	Process Consistency	Visually Clean
Drug Substance	R			R	R^S	R	R
Drug Product	R			R	R^S	R	R
API Denatured or Degraded[3]	R		A	R	R^S	R	R
IMPs[4,5]	R	A		R	R^S	R	R
Cleaning Agents	R	A				R	R
Chemicals (Non-API)	R	A				R	R

A = Alternate Approach if scientifically justified
API= Active Pharmaceutical Ingredient
HBEL= Health Based Exposure Limits (PDE, ADE)
IMP = Investigational Medicinal Product
R= Recommended Approach
R^S = Recommended if sterile medicinal product
R&D= Research data with scientifically justified limit
TTC = Threshold of Toxicological Concern

Notes:

1. The use of other approaches to determine HBEL could be considered acceptable by regulatory agencies if adequately and scientifically justified.
2. Virus, prions, and mycoplasma are addressed as part of process validation and during viral clearance studies; therefore, they are not included in this table.
3. Denatured or degraded material needs to be assessed for activity, pharmacological effects, or impact to product quality.
4. See Dolan et al., "Application of the threshold of toxicological concern concept to pharmaceutical manufacturing operations" [63]; Bercu and Dolan, "Application of the threshold of toxicological concern concept when applied to pharmaceutical manufacturing operations intended for short-term clinical trials" [64].
5. "HBEL should be established based on all available data, and particularly as the knowledge base for IMPs is continually evolving the basis for establishing HBEL, should be regularly reviewed taking into account of any new relevant data." [7]

7 Sampling

A critical component of cleaning validation is sampling. The purpose of sampling is to provide an accurate representation of the level of cleanliness of the equipment. Validating the cleaning process is done by sampling after cleaning (API or DP, cleaning agent, and bioburden as necessary) and before use (bioburden). Samples of residue are only taken from direct product contact surfaces.

As with all processes, sampling for cleaning validation must follow an approved protocol or procedure that clearly defines where and how to take the samples. Personnel taking the samples should be trained and qualified as necessary and the testing method validated over the range of testing.

The first sample for equipment is a visual inspection of the entire piece of equipment. Equipment must be VC before any other samples are taken. If the equipment is not VC, the cleaning is a failure and no further samples are taken other than as an option as part of a subsequent investigation.

One of the main steps in equipment cleaning validation is selecting the best residue detection method. There are two primary types of sampling techniques widely used in cleaning validation studies and during the routine monitoring of pharmaceutical equipment and surfaces: direct surface sampling (swabbing or contact plates) and indirect (rinsing diluent). In direct sampling, the sample is taken directly from the surface of the equipment and measured for residue levels. An example of this approach is swab sampling, which is preferred by regulatory agencies (for example, FDA [17]) because it involves the physical removal of any sample from the surface of the equipment. Although preferred, direct sampling is not always practical (e.g., pipes or hoses). In these instances, indirect sampling is the best option. In indirect sampling, the sample is taken from the surface of the equipment by a medium (e.g., water) and then the medium is measured for residue levels.

RFs for cleaning validation residue testing are an essential element of any cleaning validation test. When a cleaning sample is taken, all residue material might not be removed from the equipment. A recovery study using specified parameters is necessary to determine how much residue material is consistently recovered from equipment surfaces during sampling. This RF is then applied to cleaning samples as necessary to provide accurate cleaning data. The RF is specific for each analyte of interest (e.g., API, detergent) from each MOC. The RF may be influenced by the other recovery parameters. It is imperative from a compliance risk standpoint to limit the variability of the recovery parameters as much as possible.

This chapter reviews the different types of sampling, including advantages and disadvantages, and issues that impact the parameters of each sampling type.

7.1 Swab Sampling

Swab sampling is a critical parameter in a validated cleaning program and is essential to accurately determine amounts of residual API for a given cleaning process or equipment train [87].

Swab sampling should be conducted when:

- Access required for direct sampling is available (e.g., disassembled parts, open equipment), including use of an extension pole to reach areas (e.g., tank interiors)
- Worst-case locations can be identified and swabbed

7.1.1 Advantages and Disadvantages of Swab Sampling

The advantages and disadvantages are listed here:

Advantages

- Physical removal of residue from surfaces
- Residue is soluble in chosen solvent
- Samples taken from worst-case locations
- Small extraction volume resulting in the ability to measure lower residue amounts

Disadvantages

- Samples a small area
- Cannot access some locations (e.g., piping)
- Invasive of enclosed equipment (e.g., tanks)

7.1.2 Swab Sampling Parameters [87]

A list of swab sampling parameters is shown in Table 7.1.

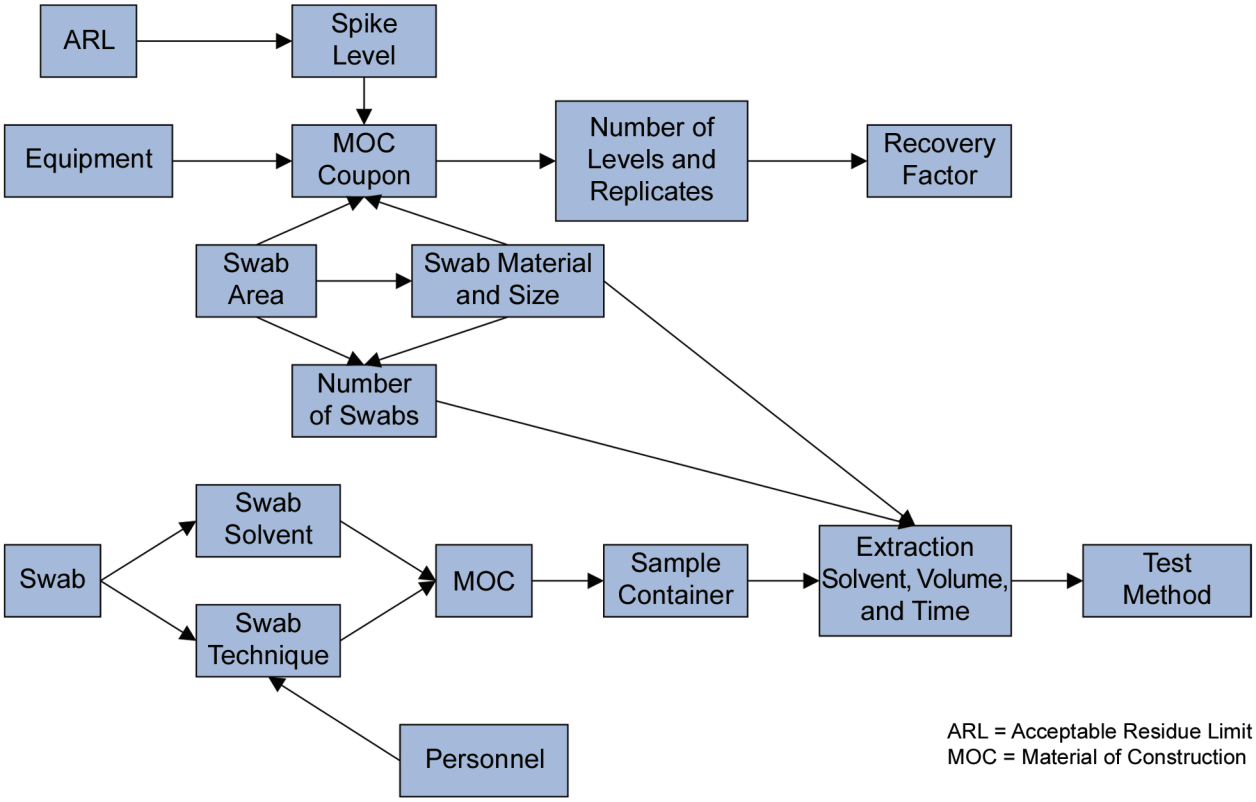
Table 7.1: Swab Parameters
Used with permission from copyright holder. Information originally appeared in Pharmaceutical Technology [87]

Coupon Material of Construction (MOC)	Residue Spike Level
Number of Spike Levels and Replicates	RF Determination
Swab Area	Type of Swab
Number of Swabs	Swab Solvent
Swab Order	Swab Technique
Personnel	Sample Container
Sample Stability (pre- and post-extraction)	Extraction Solvent
Extraction Method	Extraction Time
Test Method	Swab Sample Locations

Parameters have some flexibility and are interdependent (see Figure 7.1). Swab parameters should be consistent as much as possible across products and equipment. For example, changing the swab area between 25 cm^2 and 100 cm^2 might seem necessary for some residues, but when changing parameters, perform a risk assessment to determine whether a recovery study must be repeated.

Always wear gloves when taking swab samples. During sampling, there should be no contact with equipment surfaces other than the swabs if proper procedures are followed. And if appropriate grade swab solvents are used (PW, HPLC-grade organics), there should be no reason to reclean the equipment after swab sampling. A risk assessment should address the decision to reclean equipment or not after swab sampling.

Figure 7.1: Swab Recovery Factors and Interrelationships [87]
Used with permission from copyright holder. Information originally appeared in Pharmaceutical Technology [87]



Coupon Material of Construction (MOC)

Using coupons of the same MOC as the manufacturing or packaging equipment is necessary to provide an accurately measured level of residue recovery.

- All equipment MOCs are swabbed
- Coupons need to be the same material as the equipment, that is, the same grade of SS, plastics, elastomers. If coupons are not available or the exact MOC is not known, for example, plastic, the actual piece of equipment might be borrowed for the recovery.
- Start with SS (where SS is the majority MOC) and expand to others
- Different MOCs might be grouped based on similar RFs as demonstrated over time [88]

Residue Spike Level

The testing range of the residue spike level is typically 70%–110% of the ARL (see Chapter 6 and Chapter 8 for ARL determination). The most critical concern for accurate data is near the ARL; however, since the expectation is to clean to levels well below the ARL, extending the test range down to the LOQ can give a more representative picture of cleaning data (see Section 8.1.1.1).

- If ARL > 100 µg/25 cm^2 swab, perform recovery around 100 µg/25 cm^2
- Typical example at 70%, 100%, and 110% of ARL

- Advisable to extend down to method LOQ and one to three levels in between, which is where most data should be

Number of Spike Levels and Replicates

During swab sample method validation, a sufficient number of recovery samples should be run in order to have a statistically significant RF of a somewhat variable test. A typical example is:

- Minimum of three spike levels and all levels in triplicate on primary MOC (SS)

- Minimum of one spike level in triplicate on other MOCs that demonstrate a comparable RF

RF Determination

Example:

An example for the acceptance range for the RF determination is 70%–110% of the spiked amount, with ≤ 15% Relative Standard Deviation (RSD). Recoveries close to 100% are preferable. Lower recoveries can adversely impact the ability to pass the ARL and can raise questions as to where the unrecovered material is.

- *Investigate if outside 70%–110% range or if % RSD is > 15%.*

  - *Investigate: coupon MOC, swab material, swab technique, swab solvent, extraction solvent, extraction conditions (e.g., sonication, vortex stirring, time)*

  - *If no improvement, use data "as is." For example, if a recovery of 50% cannot be improved, then use it with the understanding that the variability will likely be higher (> 15%) and data will be less accurate. The risk of using this approach is proportional to the level of MOC presence in the equipment (e.g., gasket). A minor MOC is not a high-risk situation.*

- *Average all data that falls in the acceptance range into a single RF.*

  - *Often the % recovery drops off at low concentrations (e.g., LOQ) and high concentrations.*

  - *Using the lowest single recovery as the RF is inaccurate and risks issues with quantitation.*

See Appendix 1 for additional examples.

Swab Area

The swab should be a representative sampling of the equipment in hard-to-clean locations, which are often relatively small (e.g., valve, pipe elbow).

- Recommended swab area 5 cm × 5 cm (25 cm^2)

  - Large enough to get a representative worst-case location sample but small enough to allow multiple samples (e.g., API, detergent, bioburden)

  - Small enough to repeat accurately by eye (≥ 25 cm^2) and acceptable to go slightly over. The dimensions of the swab head can be used as a visual measuring aid on the swab area.

- Alternative swab area 10 cm × 10 cm (100 cm^2)

  - Used to increase method sensitivity (a 4x larger sample), which could be accomplished in the test method (e.g., increase injection volume, decrease extraction volume, increase flow rate)

- Hard to defend for multiple swabs of small locations or pieces of equipment
- Hard to repeat accurately without a measuring aid. For example, a ruler can be used.

- Use of a swab template (a square frame) to outline the swab area is strongly discouraged
  - Template becomes contaminated by each sample when touched by the swab
  - Sample loss occurs as swab touches template and sample wicks under the edge of the template
  - Template needs to be cleaned or changed in between each sample
  - Template cannot be used for restricted areas (e.g., valve, pipe) or non-square areas (e.g., tablet tooling)
  - Difficult to hold swab and sample vial and template simultaneously

Type of Swab [87]

Swabs and swab materials should be:

- Convenient and easy to use
- Able to pick up residue from coupon and equipment surfaces
- Able to release residues into solution
- Not cause interference with the residue assay, e.g., cotton swabs
- Made of materials that do not shed particles, e.g., do not use wooden sticks

The size of the swab head used depends on the swab area and the level of residue material on the MOC. The swab stick must be long enough so that the risk of touching the swab head is low. Ideally, the swab stick would be notched to make breaking off the swab head in the sample vial more convenient.

- Examples of swabs
  - Small polyester swabs hold 0.1 ml of solvent and pick up > 100 µg of residue
  - Large polyester swabs hold 0.5 ml of solvent and pick up > 200–300 µg of residue
- Low TOC and sterile swabs are available for TOC and bioburden testing respectively.
- Extractables from some swabs are possible in strong organic solvents (e.g., acetonitrile) over time.

Number of Swabs [87]

The swab area and the swab selection can influence the number of swabs necessary to achieve a consistent, acceptable recovery. A single swab will provide adequate recovery and require a minimal amount of extraction solvent to maximize the LOD for the residue assay and simplifies the swab process to take the sample. A larger area more often requires multiple and larger swabs to achieve a sufficient RF, but the larger volume of extraction solvent required offsets some of the sensitivity advantage gained from the increased sample size.

- It is recommended to use one swab for sampling.

- One swab should provide consistent, adequate recovery for most residues from most MOCs.

- Use multiple swabs only if one swab does not recover an acceptable level of residue, but also consider other factors for low recovery: swab solvent, swab technique, extraction solvent. If multiple swabs are used, combine them to obtain one residue level for the swab location.

Swab Solvent

The swab solvent must be one in which the analyte of interest is soluble and be compatible with the extraction solvent.

As a best practice, use a common swab reservoir from a closed container for sampling rather than pre-fill each sample container with swab solvent. Using the pre-filled sample containers makes sampling more difficult to control and spilling solvent will ruin the sample.

- The analyte of interest must be soluble in swab solvent.

- Use a volatile organic solvent (e.g., methanol, ethanol), where justified, so no solvent is left behind on equipment.

- Use water for TOC sampling.

- Do not leave swab solvent behind on the coupon or equipment after swabbing. Before swabbing, squeeze excess solvent out using the neck of the swab solvent container.

- Presoaking the swabs is typically not necessary but could be helpful if extractables from the swab are a problem.

Swab Order

The order in which multiple swabs are taken is important to prevent cross-sample contamination:

1. Bioburden samples should be taken first using aseptic techniques since they are most sensitive to any sampling contamination.

2. TOC samples should be taken before any swab samples using organic solvents since they are sensitive to any organic solvent sampling contamination.

3. Samples using organic solvents should be taken last.

Swab Technique

Use clear, easy to follow directions and a diagram for swabbing (e.g., see Figure 7.2).

- For example, swab back and forth over the swab area; flip swab; swab back and forth in a perpendicular direction over the same area.

  Note: A final swab around the perimeter may be used to pick up excess solvent or ensure complete coverage. This final swab may be of value when swabbing larger areas.

- A consistent standard swab procedure leads to minimal variability assigned to personnel.

- Following sampling, snap or cut each swab sample into a labeled sample container.

Figure 7.2: Swab Technique

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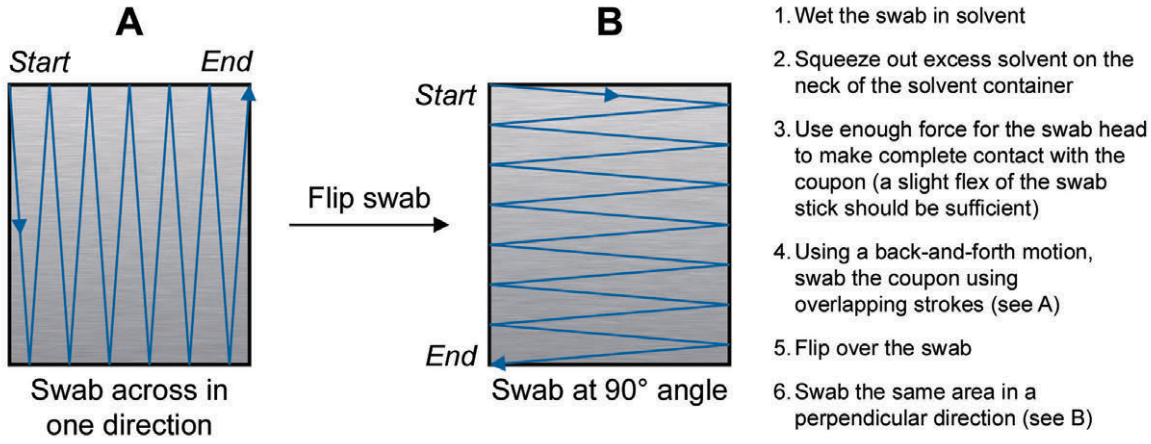


Note: Five stroke swab depicted. Other techniques are acceptable if justified (including justifying that a statistically significant surface representation is covered by the swab collection process).

If a square swab area is not available due to equipment configuration restrictions, swab the same size area over a rectangular or equivalent pattern as shown in Figures 7.3 and 7.4.

Figure 7.3: Swabbing Irregular Surfaces

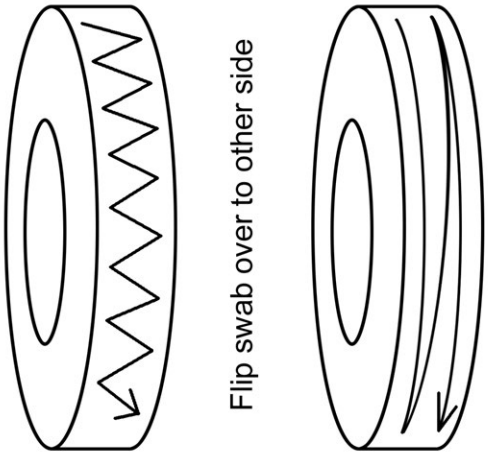
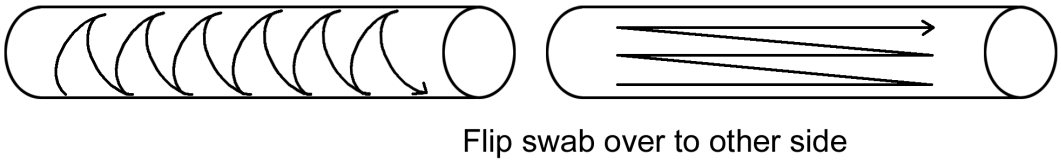


Figure 7.4: Swabbing Inner Surfaces – Process Pipe



Personnel

Personnel have always been assumed to be a major factor in the variability of swab recovery data. Although person-to-person variability is a factor, it has been shown that this variability is consistent (about 3%) across sites [88] making swab RFs transferrable among sites. Any transfer of an RF should be done formally with confirmatory swab recovery at the receiving site.

- Swab technique should be emphasized during training and swab qualification
- Ensure that all personnel use the accepted technique
- Qualify personnel if other equipment (e.g., extension pole) are used for sampling certain pieces of equipment
- Qualify personnel with at least triplicate recovery at one level of a chosen residue using the swab, solvent, and surface area used for sampling. Acceptance criteria for swab qualification needs to be established. For example, the average of the uncorrected three recoveries are within ± 10% of the established RF with a variability of ≤ 15% RSD.

Sample Container

The sample container must be large enough for the volume of the extraction solvent, be compatible with the extraction solvent, and not contain anything that might be extractable by the solvent or a source of contamination in the extracted sample.

Once samples are collected, accurately add extraction solvent to the sample container.

- Use inert plastic or glass containers of sufficient size
- Beware of lid liners for extractables

Sample Stability (Pre and Post-Extraction)

Establish sample stability on the swab before extraction and then of the extracted solution until analysis. Samples on the swab can dry out and become more difficult to extract or the analyte of interest could degrade. Sample solution stability is a standard component of analytical method validation. Ideally, coordination between the swab sampling and the testing laboratory minimizes both periods of time. See Section 8.1.1.9.

Extraction Solvent

The extraction solvent needs to completely dissolve and recover the analyte of interest from the swab head.

The extraction solvent can often be the same as the swab solvent but does not need to be. The swab solvent must dissolve the analyte of interest. The extraction solvent must be compatible with the assay method. Using one solvent might not be the best way to address both concerns. For example, the swab solvent might be organic but the extraction solvent could be an organic/aqueous mixture.

- Extraction solvent is compatible with the test method and dissolves the API or detergent
  - TOC solvent must be water, dilute acid, or dilute base
- Volume of extraction solvent
  - Smaller volume concentrates sample

- TOC typically 20–40 ml water
- HPLC can be 10 ml or less
- For small swab head and HPLC, volume could be as little as 2 ml
- Use lowest practical volume to maximize sensitivity

- Based on ARL: the lower the ARL, the smaller the extraction solvent volume needed for maximum sensitivity
- Sensitivity of test method: the more sensitive the LOQ of the test method, the larger the extraction solvent volume can be used

Extraction Process

The recommended strategy is to match the extraction technique and time with the anticipated hold time for the swab samples prior to extraction, and apply the sample extraction parameters to all cleaning validation swab samples for consistency.

The most common swab extraction techniques are: vortex mixing, shaking, or sonicating, all of which can be effective.

- Vortex mixing is meant for relatively short hold times (generally, for a minute or less per sample) since the samples are held either individually, in groups, or racked and vortexed.
- Mechanical shaking is for longer hold times but samples generally must be individually attached to the shaker or put into a rack and the rack attached to a shaker table.
- Sonication is for longer hold times and sample manipulation is minimal since samples are placed into a rack and the rack placed in the sonicator.

The technique chosen is often based on convenience, that is, what is readily available and how many samples are to be handled at once.

The extraction method chosen can be influenced by the swab solvent and how long samples sit before extraction; the more volatile the swab solvent and the longer samples sit, the more likely they are to dry out and the more rigorous extraction method is needed.

For consistency, choose one extraction method and maintain for all recoveries.

Extraction Time

Extraction time is tied to the extraction method and the swab solvent used, and can be influenced by how long samples are held before extraction. As with the extraction method, the more volatile the swab solvent and the longer samples sit, the more likely they are to dry out and the more extraction time is needed.

During method development, time can be varied to determine optimal recovery.

Test Method

The test method must be validated and sensitive enough to measure samples at levels lower than the ARL (see Chapter 8). A specific method (e.g., HPLC) is preferred but a non-specific method (e.g., TOC) is acceptable where appropriate. Bioburden testing is often conducted using compendial methods as a general test, and then individual species identified as necessary.

As much as possible, use methodology with which the analysts are experienced.

- Pharmaceutical laboratories generally use HPLC test methods
- Biopharmaceutical laboratories generally use a TOC test method
- Most detergent suppliers are able to provide either an HPLC or a TOC test method

Validate the test method to quantitate levels well below the ARL as much as possible, including the LOQ and LOD, so that data can demonstrate control of the cleaning process.

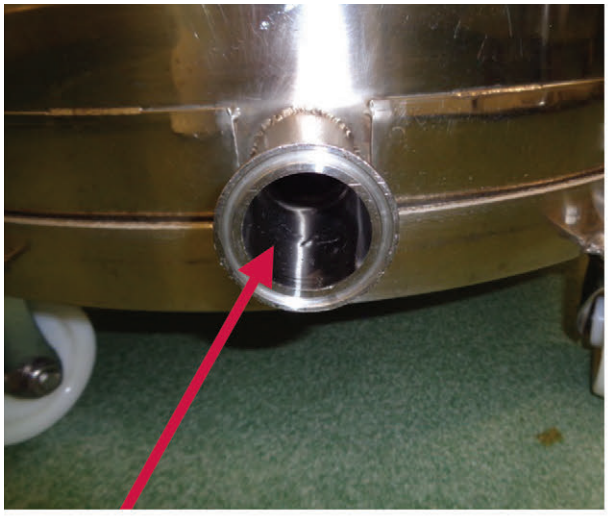
Swab Sample Locations

The ARL calculation assumes that any residue left on equipment product contact surfaces is distributed uniformly over the equipment. This assumption is only reasonable if the swab sample locations are those that will be the first locations to accumulate residue, the last locations to lose residue during cleaning, or are otherwise hard to clean. The number of swab samples (suggested 2–7) should reflect the size and complexity of the equipment and provide a representative picture of the cleanliness of the cleaned equipment.

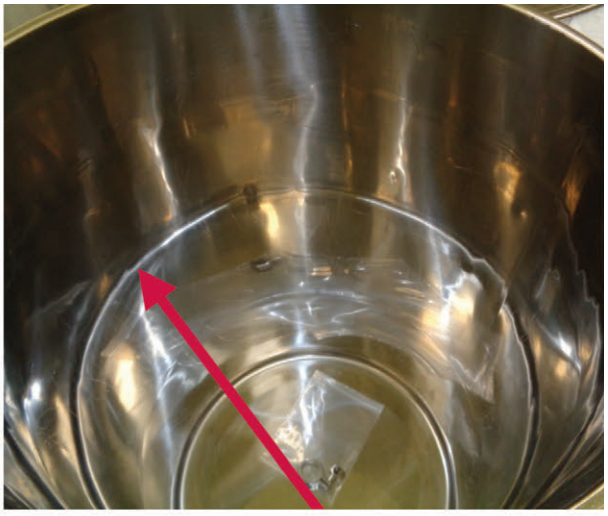
Based on cleaning failures, more frequent testing should be done based on increased risk rather than increasing the number of swab locations. Also, some facilities reduce the number of locations after successful testing, but any sample reduction needs to be justified. A better approach is to have a set number of samples and either increase or decrease the frequency of sampling based on risk.

These worst-case locations are based on equipment geometry, complexity, degree of disassembly, and the cleaning method used. All MOC from the worst-case swab locations are noted for swab recoveries. All the parameters that go into a reliable swab sample are rendered meaningless if the samples are not taken in the right location. It is highly recommended to include drawings or pictures of the swab locations to ensure consistency of sampling as shown in Figure 7.5. Examples of worst-case swab locations and their rationale are presented in Table 7.2, and examples of specific equipment locations are given in Table 7.3.

Figure 7.5: Swab Locations



Discharge Port



Side of Tank at Fill Line

Table 7.2: Typical Worst-Case Swab Locations Rationale

Swab Location	Rationale									
	Hard to Clean	Constant Product Contact	Product Buildup	Residue Buildup	Seam	Pinch Point	Hard to Reach	Hard to Sample	Contamination Risk	Irregular Shape
Corners	x									
Valves		x	x		x					
Interface between Two Surfaces (Gaskets)		x	x		x					
Mixing Blades		x				x				
Tablet Press Tooling; Filling Needles ("hot spots")									x[1]	
Sides of Hoppers, Containers or Tanks		x					x[2]			
Hoses or Pipes	x							x		
Screens	x									x
Liquid/Air Interface, Side of Tank	x			x						
Tank Port							x			
Blind, Shadowed or Occluded Spot (e.g., blocked by mixing blade shaft in a CIP process)	x									
Drains		x	x							x

Notes:
1. Risk of contaminating a limited number of doses
2. Large Equipment

Table 7.3: Worst-Case Swab Locations by Equipment Type

Equipment	Swab Location	Rationale										
		Hard to Clean	Product Contact	Product Build-Up	Seam	Pinch Point	Hard to Reach	Contamination Risk	Irregular Shape	Can Retain Water	Liquid/Air Interface	Occluded Location
SS Bin	Butterfly Valve		x	x								
	Wall		x				x					
	Neoprene Discharge Port Gaskets			x	x							
Mixing Tank	Bottom Surface of the Tank		x				x			x		
	Wall		x				x				x	x
	Lid Tank Port	x										
	Mixer Shaft		x				x		x		x	
	Mixer Blade		x				x		x		x	x
	Discharge Tank Port	x	x		x				x			
	Vortex Breaker	x	x		x		x					
Reactor	Bottom Surface of the Tank		x				x			x		
	Wall		x				x				x	x
	Lid Tank Port	x										
	Mixer Shaft		x				x		x		x	
	Mixer Blade		x				x		x		x	x
	Baffle	x	x		x				x			
	Discharge Tank Port	x	x		x				x			
Filter	Filter Housing		x				x			x		
	Filter (depending on MOC)	x	x	x					x			
Centrifuge	Scraper	x	x			x			x			
	Top Basket	x	x						x			
	Discharge Chute	x	x						x			
	Bottom Chamber		x				x			x		

Table 7.3: Worst-Case Swab Locations by Equipment Type (continued)

Equipment	Swab Location	Rationale										
		Hard to Clean	Product Contact	Product Build-Up	Seam	Pinch Point	Hard to Reach	Contamination Risk	Irregular Shape	Can Retain Water	Liquid/Air Interface	Occluded Location
Printer	Wall of Hopper		x						x			
	Discharge Chute		x						x			
Deduster	In-Feed Chute		x						x			
	Perforated Coil Near Inner Shaft		x						x			
	Discharge Chute		x						x			
Metal Detector	In-Feed Chute		x						x			
	Discharge Chute		x						x			
Double Cone Blender	Lid		x		x							
	Upper Wall		x				x					
	Lower Wall		x				x					
	Discharge Port		x		x	x			x	x		
	Vortex Breaker	x	x		x		x					
Film Coater	Pan Front		x				x					
	Pan Back		x				x					
	Pan Screen		x						x			
	Baffle		x				x		x			
	Coating Solution Container (detergent only)								x	x	x	
Mill	Hopper		x						x			
	Bottom Chute		x						x			
	Milling Blades		x			x			x			
	Screen	x	x						x			

Table 7.3: Worst-Case Swab Locations by Equipment Type (continued)

Equipment	Swab Location	Rationale										
		Hard to Clean	Product Contact	Product Build-Up	Seam	Pinch Point	Hard to Reach	Contamination Risk	Irregular Shape	Can Retain Water	Liquid/Air Interface	Occluded Location
Fluid Bed Dryer	Product Container		x				x					
	Agitator Rake at the Bottom of the Product Container	x	x			x						
	Sampling Port Located on the Product Container	x	x						x			
	Filter Plate	x	x						x			
	Inner Surface of the Wurster Column		x						x			
	Entire Surface of the Nozzle Tip	x							x		x	
Hand Screens	Surface of the Screen	x	x	x	x							
	Inside Edge of the Screen and Wall	x	x		x				x			
Planetary Mixer	Bottom of the Bowl		x						x	x		
	Side of the Bowl		x				x					
	Agitator Blade		x			x			x			
Tablet Press	Hopper		x				x					
	Adjustable Chute		x						x			
	Star Wheels	x	x						x			
	Feed Frame		x						x			
	Turret						x		x			
	Discharge Chute		x						x			
	Tooling	x	x					x	x			
Mixing Blade	Blade Head	x							x			
	Blade Shaft								x			

Table 7.3: Worst-Case Swab Locations by Equipment Type (continued)

Equipment	Swab Location	Rationale										
		Hard to Clean	Product Contact	Product Build-Up	Seam	Pinch Point	Hard to Reach	Contamination Risk	Irregular Shape	Can Retain Water	Liquid/Air Interface	Occluded Location
Roller Compactor	Lower Hopper		x				x					
	Left and Right Rollers		x			x			x			
	Roller Housing		x				x					
	Screen	x	x	x	x							
V-Blender	Top Port, Near the Welds		x				x					
	Right Arm, Down from Top Port		x				x					
	Left Cover, Near the Welds		x						x			
	Discharge Port Butterfly Valve	x	x		x	x			x	x		
	Neoprene Discharge Port Gaskets	x	x		x							
	I-Bar Shaft	x	x						x			
	Vortex Breaker		x		x		x					
High-Shear Granulator	Inside the Bowl in the Corner	x	x							x		
	Impeller Blade	x	x						x			
	Chopper Blade	x	x						x			
	Wall of Discharge Chute		x						x			

7.1.3 Swab Sampling Recovery Execution

Once all the swab recovery parameters are established, the recovery study can be executed (see the example in Figure 7.6). Always run a blank sample to determine whether any potential interference (e.g., extra peaks) is not related to the analyte of interest. As shown in Figure 7.6, additional blanks (e.g., swab, swab plus swab solvent) can be tested to refine an investigation into extra peaks. Recovery best practices are given in Table 7.4. Recovery execution examples are presented in Appendix 1 and Appendix 2.

Figure 7.6: Swab Sample Recovery Study [87]

Used with permission from copyright holder. Information originally appeared in Pharmaceutical Technology [87]

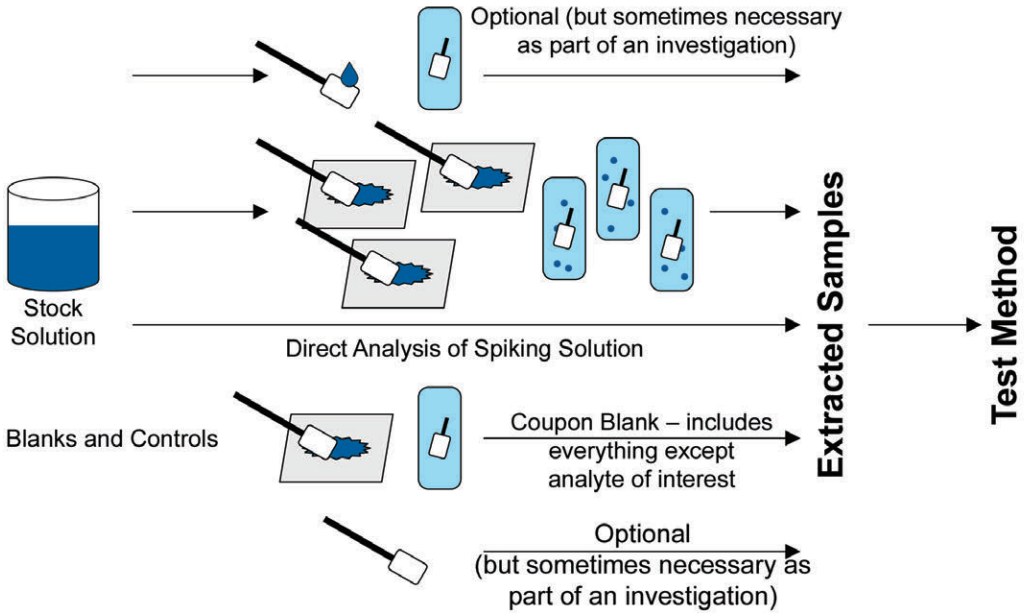


Table 7.4: Swab Recovery Best Practices [87]

Used with permission from copyright holder. Information originally appeared in Pharmaceutical Technology [87]

Parameter	Best Practice
Materials of Cconstruction (MOC)	Stainless steel coupons Others as necessary after grouping
Spike Level	Around Acceptable Residue Limits (ARL). Down to Limit of Quantitation (LOQ) if practical
Number of Levels and Replicates	Minimum three levels in triplicate. LOQ if practical
Recovery Factor	Average of all recoveries with % Relative Standard Deviation (RSD) ≤ 15%
Swab Area	25 cm^2
Swab Material and Size	Small woven polyester swab or equivalent
Number of Swabs	One unless more are necessary
Swab Solvent	Dissolves analyte of interest; enough to wet swab and not leave liquid on coupon
Swab Technique	Back and forth; flip swab; back and forth in perpendicular direction
Swab Personnel	Personnel establish recovery factors or are qualified separately
Sample Container	Large enough for extraction; does not contribute extra peaks
Extraction Solvent	Dissolves analyte of interest; compatible with assay method
Extraction Technique and Time	Match the extraction technique and time to the anticipated hold time for the swab samples
Test Method	Select a method for familiarity and experience. Validate the method using all validation parameters

7.2 Rinse Sampling

Rinse sampling is one of the methods used to test for residual contamination following a defined cleaning process as stated by the EU [4], FDA [17], and PIC/S [23]. It is important to note that there may be circumstances where direct sampling methods are not possible and/or impractical, and thus rinse sampling may be the preferred alternative:

PIC/S [23]:

> *"There are two methods of sampling that are considered to be acceptable, direct surface sampling (swab method) and indirect sampling (use of rinse solutions). A combination of the two methods is generally the most desirable, particularly in circumstances where accessibility of equipment parts can mitigate against direct surface sampling."*

FDA [17]:

> *"There are two general types of sampling that have been found acceptable. The most desirable is the direct method of sampling the surface. Another method is the use of rinse solutions."*

EU [4]:

> *"Sampling should be carried out by swabbing and/or rinsing or by other means depending on the production equipment. The sampling materials and method should not influence the result. Recovery should be shown to be possible from all product contact materials sampled in the equipment with all the sampling methods used."*

Rinse sampling is an example of indirect sampling, as any remaining surface residue is not taken directly from the equipment surface. It is performed by collecting an aliquot of either the final rinse or a separate sampling rinse from a piece of equipment or equipment train after cleaning. The absence of residue in the rinse sample infers an absence of residue on the actual surface.

As in swab sampling, the residue assays are validated for the following parameters: linearity, precision, sensitivity, specificity, LOD, and LOQ. Rinse RFs for residue testing are also required (see Section 8.1.1).

Although it is possible to use rinse samples only for cleaning validation studies, it is preferred that these samples are taken in combination with a direct sampling method such as swab sampling. Rinse sampling on its own can be used for Continued Cleaning Verification (Stage 3) or cleaning monitoring.

FDA December 1998 Human Drug CGMP Note [89]:

> *"While it is understood that rinse samples are capable of sampling larger surface areas, particularly ones which are difficult to access, for the purposes of cleaning validation, rinse samples alone would not be acceptable unless a direct measurement of the residue or contaminant has been made. One disadvantage of rinse samples is that the residue or contaminant may not be soluble or may adhere to the equipment. Some firms use both swab samples, where feasible, and rinse samples during the course of their cleaning validation."*

7.2.1 Advantages, Disadvantages, and Limitations of Rinse Sampling

Advantages of Using Rinsing Sampling

- The FDA Guidance [17] states:

  "Rinse Samples – Two advantages of using rinse samples are that a larger surface area may be sampled, and inaccessible systems or ones that cannot be routinely disassembled can be sampled and evaluated."

- It maintains system closure (sampling technology should not contaminate or cause contamination of the sample)

- Easier and simpler than direct sampling
- Reduced number of samples required
- May use a different solution from process and/or final clean rinse
- Provides overall picture
- Samples inaccessible systems or ones that cannot be routinely disassembled
- Less technique dependent (more simplistic) than swabs
- Adaptable to online monitoring
- Allows sampling of unique (e.g., porous) surfaces
- Can adjust rinse volumes to give a consistent acceptance criterion
- Analysis can be online or off-line
- Direct sampling cannot be carried out safely
- Interventions into cleaned equipment could contaminate the equipment
- Equipment or surface areas cannot be easily accessed for swabbing (such as pipelines, wire mesh, or transfer hoses)
- Equipment would have to be dismantled for direct sampling during validation, and then not disassembled for routine monitoring
- Equipment size makes direct sampling difficult or impossible (e.g., filling needles)
- Worst-case locations cannot be accessed or access is restricted
- Small or difficult to reach surface areas
- Access required for direct sampling is difficult or impossible (e.g., for sealed equipment, hard welded pipework)

Disadvantages

- May not be acceptable as the sole method of testing per the FDA [17]. A disadvantage of rinse sampling is given as:

  "…the residue or contaminant may not be soluble or may be physically occluded in the equipment."

  Furthermore, for rinse sampling:

  "Check to see that a direct measurement of the residue or contaminant has been made for the rinse water when it is used to validate the cleaning process. For example, it is not acceptable to simply test rinse water for water quality (does it meet the compendia tests) rather than test it for potential contaminates."

  The FDA uses the analogy of a dirty pot [17]:

  "In the evaluation of cleaning of a dirty pot, particularly with dried out residue, one does not look at the rinse water to see that it is clean; one looks at the pot."

- Need to correlate amount of residue detected or present in the rinse sample to the potential contamination of next product
- The residue or contaminant may not be soluble
- The equipment/process used should suitable to be wetted (i.e., milling, mixing, filters etc. may not be suitable for rinse sampling)
- The rinse solvent should be the same as the final rinse medium (e.g., water or solvent)
- The residue or contaminant may be physically occluded in the equipment
- Rinse volume is critical to ensure interpretation of results
- It requires control over the solvent used for rinsing, the contact time, and mixing involved
- Deficiencies associated with rinse sampling relate mainly to the uncertainty in the RF, as well as the rinse itself, which dilutes any contamination present on the equipment surfaces (thus, there is the need to correlate the amount of residue detected or present in the rinse sample to the potential contamination of next product)
- Limited information about the actual surface cleanliness in some cases
- May lower test sensitivity through dilution of the analyte of interest
- Inability to detect the location of residues
- Rinse volume is critical to ensure an accurate interpretation of results
- Sampling methodology must be defined since the rinse sampling method and location can influence results
- May be difficult to accurately define and control the areas sampled; therefore, it is usually used for rinsing an entire piece of equipment, such as a vessel
- Reduced physical sampling of the surface
- Samples taken from a recirculatory rinse may also include residues from the CIP skid and/or supply and return lines

7.2.2 Requirements for Rinse Sampling

In order for rinse sampling to be a viable sampling method:

- Solvent should dissolve the target residue
- Rinse solvent should reach all product contact surface areas
- Surfaces should be rinsed long enough to ensure complete coverage and sufficient removal of the target residue

7.2.3 Rinse Sample Parameters

- Coupon MOC
- Type of rinse sample, e.g., final rinse sample (i.e., last rinse of cleaning process) or separate post-cleaning sample rinse

- Rinse solvent
- Residue spike level testing range
- RF determination acceptance range
- Volume of rinse

7.2.4 Sample Recovery Testing

As with direct sample techniques, sample RFs need to be established. There are several ways that this can be performed.

The chosen MOC surface sample coupon is spiked with a known amount of the target residue and usually is allowed to dry. The chosen solvent (usually water) is the same quality and temperature as that used for the equipment final rinse.

Note: Test coupons need to be the same MOC as the equipment being tested.

As a representation of worst-case rinse recovery conditions, the solvent is allowed to flow over the test coupon (using a pipette or similar tool) and collected (see Figure 7.7). It is difficult to simulate the impingement force or agitation of the solvent flow and the volume of solvent used should be limited and controlled to ensure a rinse recovery sample concentration that is within the validation parameters of the test method.

Figure 7.7: Rinse Sample Recovery Study
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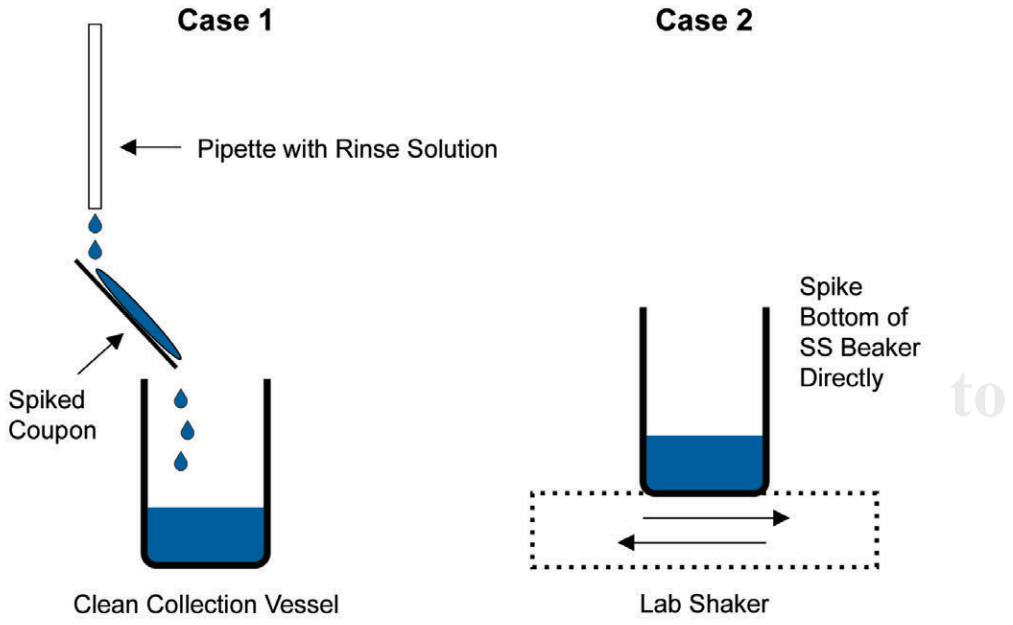


Alternatively, a container (e.g., beaker) made from the same MOC as the equipment being tested can be used – the bottom of the container is spiked with the test residue and allowed to dry. The calculated solvent rinse volume is then added to the container and swirled or shaken for a specified time before pouring off the solvent for analytical testing. If a suitable MOC container cannot be obtained, a spiked MOC coupon can be placed in the bottom of a normal container and the calculated volume of solvent added as before.

Whichever recovery test method is chosen, it is important to ensure that blank and control samples are tested.

If an MOC coupon is not available, spike recovery testing can be performed on the piece of equipment.

Coupon MOC

Different materials used in the construction of equipment items can have different cleaning efficiencies, and return different RFs. As such all MOC to be sampled in the equipment items/train should be identified, tested, and RFs calculated for each of them. Establish and use an RF for each MOC or as with swab testing, there might be the potential to group MOC based on accumulated recovery data [88].

Residue Spike Level Testing Range

Spike concentrations should be chosen to bracket the ARL (suggested 50%, 100% and 150% of ARL). However, since the expectation is to clean to levels well below the ARL, and the rinse itself will have diluted the residue to a level below that which would be seen using a direct sampling method, extending the test range down to the LOQ can give a more representative picture of the cleaning data. Recoveries at levels close to the analytical method LOQ can result in lower than expected recoveries and with high % RSDs.

The recovery levels may also be affected by the volume of the rinse water used; too small a volume will not remove the residue and too large a volume dilutes the residue to the point that it may be undetectable [90].

Number of Spike Levels and Replicates

A sufficient number of recovery samples should be run in order to have a statistically significant RF.

- Best practice is to use a minimum of three spike levels and all levels in triplicate on primary MOC (usually SS) should be measured
- A minimum of one spike level in triplicate on other MOCs
- Average all data that falls in the acceptance range into a single RF

Note 1: Often the % recovery drops of at low concentrations (e.g., LOQ) and high concentrations.

Note 2: Do not use the single lowest recovery number for the RF. It is not statistically representative of the data.

RF Determination Acceptance Range

As in swab sampling, recoveries close to 100% are preferable, but it is acceptable if the RFs are 70%–110% of the spiked amount, ≤ 15% RSD. If recovery values or RSD values are outside these ranges, then they should be investigated.

Types of Rinse Samples

There are two methods of obtaining a rinse sample:

- Taking a grab sample from the end of the final solvent rinse (i.e., last rinse of cleaning process)
- Performing a separate sampling rinse after the completion of the normal cleaning rinse

If a sample is taken from the end of the final rinse cycle, then it is normally taken before the very end of the rinse to ensure that a sufficient sample volume is obtained. In this case, it could be considered the worst-case sample.

If a separate sampling rinse is employed, the volume of liquid used to rinse the equipment should be determined. The volume needs to be shown to be sufficient to cover all product contact surfaces of the equipment.

While a separate sample rinse can use a different solvent from that used for the normal cleaning cycle (perhaps to demonstrate that the normal cleaning removed all of the residue), a separate sampling rinse using a different solvent may also be regarded as an additional cleaning stage; therefore, this method of obtaining a rinse sample should be used only after full consideration.

Volume of Rinse

The minimum amount of solvent should be used to avoid unnecessary dilution of the sample. A general starting point is a volume between 0.5–1 ml/cm^2, but it is strongly dependent from how intricate the sampled surface is (e.g., a wire mesh requires far higher volumes than a SS filling needle).

In determining the volume of equipment rinse to use, the following factors should be taken into account:

- The volume of rinse solvent (or method of delivery) should be sufficient to ensure solvent contact with all product contact surfaces.
- The solvent should make contact with the product contact surface for a sufficient time to dissolve any residual material.
- A high rinse volume may dilute the sample below the LOQ of the analytical method.

In calculating the volume of rinse solvent used, it should be remembered that the amount of solvent used in the rinse is not recovered 100% due to losses by evaporation and solvent remaining on the sampled surface.

The ratio of rinse solvent volume used per surface area of the equipment being cleaned should be calculated and used in the rinse sample recovery studies as shown in Figure 7.7.

7.2.5 Rinse Solvent

The final rinse and any post-clean rinse solvents are usually water; however, any solvent can be used. There is no requirement for the post-clean rinse to be the same solvent as the final cleaning rinse. Of course, if TOC testing is to be performed, the rinse solvent must be water.

The rinse solvent(s) used should:

- Have a high solubility of the product to be removed. If a post-clean rinse is used, the solvent should have an equal or higher solubility as the final clean rinse for the target residue.
- Not degrade the product
- Be compatible with the equipment
- Not cause an environment hazard
- Not interfere with or affect the subsequent residue analysis
- Either simulate a subsequent batch or at least not be a contaminant of subsequent batch

Hazardous solvents (benzene, ethylene dichloride, etc.) should not be selected as cleaning agents or for post-cleaning rinse solution.

7.3 Placebo Sampling

Although not recommended, another possible method is placebo sampling. It is a rarely used method to detect residual contamination of equipment by processing a placebo batch subsequent to the cleaning process.

In Section VI of the FDA Inspection of Validation of Cleaning Processes [17], the FDA expresses concerns that using placebo product to validate cleaning processes:

- *"One cannot assure that the contaminate will be uniformly distributed throughout the system"* and that the contaminant residue may not be uniformly dispersed in the placebo.
- It cannot be assumed that a residual contaminant would be worn off the equipment surface uniformly.
- *"Analytical power may be greatly reduced by dilution of the contaminate."*

As such the FDA Guidance [17] states:

> *"Rinse and/or swab samples should be used in conjunction with the placebo method."*

The WHO [91] and Canada [13], among other agencies, also state that the batch placebo method should be used in conjunction with rinse and/or surface sampling method(s).

Because of the technical challenges, difficult justification, and the risk of potential agency challenge of the technique, placebo sampling is discouraged.

7.4 Sampling for Bioburden and Endotoxins

For surface contaminants such as microorganisms and endotoxin, it is critical to validate the sampling methods (direct and indirect surfaces) in combination with the chosen testing method so that microorganisms are not introduced into the manufacturing process. The swab technique typically involves moistening a swab with the validated extraction solution to sample a measured area in a systematic manner; whereas, the use of contact plates involves touching growth media on the surface area of equipment.

There are commercially available cleaning validation kits specifically designed for swabbing surfaces for both endotoxin and bioburden; however, surface contact plates are only effective in recovering microorganisms. Limits for endotoxins and bioburden should be established for both rinse and surface samples. The recovery studies for endotoxins and microorganisms need to be complete before evaluating the efficiency of the cleaning process. In the absence of such a validation study, a manufacturer may inaccurately assume that the equipment is clean, based on negative results.

In developing a sampling plan for cleaning validation studies, it is important to understand the limitations of the sampling method sensitivity relative to the surface to be sampled. The selection of the sampling and test method must be a scientifically sound procedure that can be validated for its intended use. For example, swab surface sampling is much better for small and irregular surfaces (filling needles, gaskets, etc.), whereas contact plates surface sampling is much better for flat and larger surfaces (process tanks, vessels, etc.).

7.4.1 Cleaning Risk Assessment for Biological Contaminants

Prior to performing any cleaning process steps (laboratory recovery studies, development and cleaning validation), a risk assessment is conducted. The rationale is to determine the risk to the product based on an understanding of the process as well as scientific knowledge that links the risk to product quality and patient safety. This section describes contaminants related to bioburden and endotoxins only.

The level of risk should be based on the following:

- How effective is the cleaning process and are the CPPs that have been established based on scientific knowledge?
- What are the hazards associated with the cleaning process? Process residues, carryover, contaminants, etc.
- Does the detection method effectively determine the residual limits that may affect the final product quality?
- Can all the equipment surfaces be effectively visually inspected?
- Can equipment surfaces be accurately sampled for contaminants such as endotoxin and bioburden?
- Is there an effective sampling method that allows the determination of an accurate residual limit for contaminants?
- What is the microbial/endotoxin impact of product contact surfaces during DHTs?
- What is the impact of bioburden and endotoxin levels during CHTs?
- How effective is the drying time in reducing residual water?

An important factor to consider before cleaning validation can be performed is: is it necessary to perform bioburden and endotoxin sampling and monitoring?

Critical aspects of microbiological sampling and monitoring are the different types of:

> *"microorganisms themselves (a direct hazard) and the presence of residues that potentially provide a microbial growth source, should contamination be present or contamination occur during the hold period (an indirect hazard)."* [92]

Other factors to consider are:

- Process or product types
- Upstream and downstream processes
- Dirty and clean equipment hold times
- Liquid and powder products
- Sterile and non-sterile products
- Biological versus traditional pharmaceutical; for example, bioburden limits for non-sterile products are typically much higher than sterile products. Also, the establishment of endotoxin limits for non-sterile products is not a concern whereas they are extremely important for sterile products.

To evaluate these microbiological risks, a logical and rational approach should be used when developing the microbiological sampling plan. In a chemical assessment, equipment may be soiled with a residue material, which is sampled and tested to evaluate the cleaning effectiveness. However, microbial contaminants cannot be introduced, making the microbial sampling process critical [92].

Cleaning validation for microbial assessment should use a risk assessment strategy that identifies the types of hazards, and risk assessed, centered on the severity of the hazard and the likelihood that the hazard will arise. Typically, microorganisms represent a hazard if they are exposed to direct product contact surfaces. Process Failure Mode Effect Analysis (pFEMA) and HACCP are very useful tools that can identify, and correct hazards associated with potential microbial contaminants. However, HACCP is best suited for microbiological risk analysis.

Microbiological hazards to consider are:

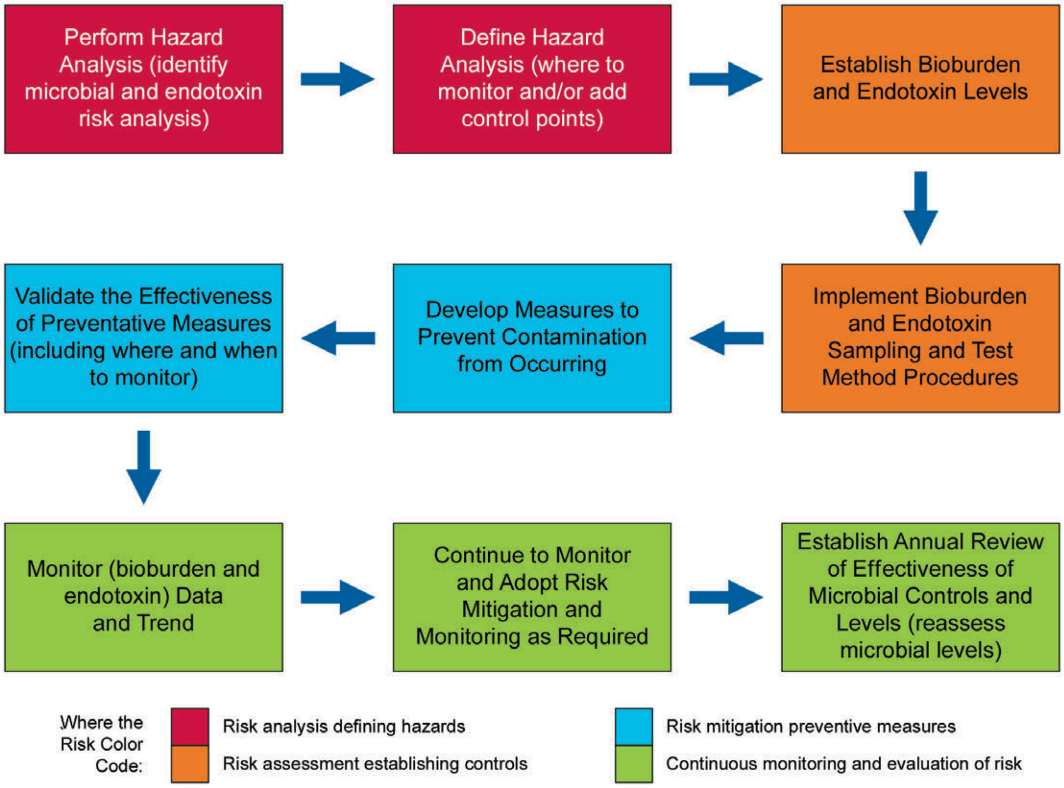
- CHT if there is concern with microbiological risk
- DHT because the potential of microbiological proliferation could impact the cleaning process efficiency (in relation to microbial proliferation and the release of endotoxin from Gram negative organisms)
- Storage of the equipment post cleaning prior to utilization or following sanitization or sterilization step
- Types of equipment product contact surface material (some types of microbials will not survive on certain material surfaces because of antimicrobial properties)
- Types of raw material and product (some types of microbials will not survive in raw material and product because of antimicrobial properties)
- Microbial proliferation from the storage environment and equipment (age of equipment could cause microbiological proliferation)
- Personnel and environmental exposure to equipment product contact surfaces
- Microbial contamination on the equipment direct surface contact post-use (and pre-cleaning)
- Efficacy of the cleaning process (cleaning agent, etc.) to remove microorganisms and endotoxin

Additionally, the risk assessment should consider:

- The severity should a level of microorganisms be present
- The likelihood of microbial contamination still being present after a cleaning and storage step, which is affected by the equipment design, environment, personnel interaction, and easiness of cleaning

The HACCP risk assessment process flow diagram for microbial contamination hazards during cleaning validation are defined Figure 7.8.

Figure 7.8: Establishing Hazard Analysis and Risk Assessment Strategy using HACCP



Furthermore, when establishing limits for bioburden and/or endotoxin, there are risk factors from the cleaning process to assess. Table 7.5 describes a few examples.

Table 7.5: Cleaning Risk Factors

Cleaning Risk Factor	Rationale
Pre-Cleaning versus Post Cleaning	There should be an evaluation of process capability by comparing the endotoxin and bioburden levels before (pre-cleaning after production dirty surface, initial rinse cycle) and after (post cleaning after final WFI/PW rinse cycle) cleaning.
Swab Method versus Contact and Rinse Samples	Direct surface sampling using swabs and contact plates are more effective recovery methods than rinse sampling. However, rinse sampling is important for surfaces that cannot be sampled using direct method, i.e., process piping where it is impossible to get a representative sample using a direct method.
Upstream versus Downstream Processes	An evaluation of the cleaning process capability for downstream and upstream should be performed. Typically, acceptance criteria for endotoxins and bioburden levels are less stringent for downstream processes than upstream processes.
Inhibition versus Enhancement of Test Samples	An evaluation should be performed to determine if the cleaning agents, product, or intermediates can cause inhibition (bioburden/endotoxin) or enhancement (endotoxin) of the sample test results.
Antimicrobial Properties of Product or Cleaning Agents	An evaluation should be performed to determine if the cleaning agents interfere with the sampling method's ability to recover endotoxin and bioburden.

Table 7.5: Cleaning Risk Factors (continued)

Cleaning Risk Factor	Rationale
Surface Material Types (stainless steel versus rubber gaskets)	Different surface materials have a direct impact on the recovery method. During bioburden/endotoxin recovery studies, the effectiveness of the sampling method should be challenged using different materials. Some materials will have a direct impact on low recovery values. Based on low recovery results, sampling methods may differ for different surface materials.
Worst-Case Sampling Locations	During cleaning development and process analysis studies, worst-case sampling locations should be established. These locations should be sampled during cleaning validation studies.
Process Mapping	Process mapping should be performed to determine all locations requiring manual, semiautomatic, and automatic cleaning. At this stage, each cleaning process step should be identified.
Dirty Hold Times (DHT) and Clean Hold Times (CHT)	During process mapping, DHTs and CHTs should be developed and validated. Typically, DHT should not exceed 24 h because of concerns with microbial growth. CHT should be validated at worst-case times.
Test and Sample Method Selection	A risk assessment should determine the best sampling and testing methods for microbial and endotoxin contaminants.

All of these risk factors need to be evaluated to help ensure product quality and patient safety have not been compromised.

7.4.2 Bioburden Sampling Methods Consideration

Surface microbial bioburden monitoring methods are described in the literature.[16] Research[17] has also indicated that:

> *"the contact plate method is suitable for flat, firm surfaces, (considering both recovery and repeatability), whereas swabbing is better for flexible and uneven surfaces and for heavily contaminated surfaces."* [95]

However, the use of contact slides for the sampling of irregular surfaces has shown to be a very effective sampling method in the recovery of low-level bioburden.

There are various sampling techniques used to confirm microbial cleanliness and characterize the product bioburden. Sterile swabs and/or contact plates from surface samples and rinse samples can be used as one sample method for generating samples for microbial testing. Methods of microbe isolation and identification can be the same ones routinely used in the microbiology laboratory. Cleaning agents should be checked to identify their level of bioburden, if any. Whatever method is selected for the bioburden recovery must be validated [17].

In addition, analytical test methods need to be consistent with the FDA cleaning guidance document [17].

> *"The firm should challenge the analytical method in combination with the sampling method(s) used to show that contaminants can be recovered from the equipment surface and at what level, i.e., 50% recovery, 90%, etc. This is necessary before any conclusions can be made based on the sample results. A negative test may also be the result of poor sampling technique."*

[16] See Dyer et al. [93], as cited in [95].
[17] Information from Niskanen and Pohja [94], as cited in [95].

7.4.2.1 Cleaning Validation Studies Consideration for Bioburden and Endotoxin

Some of the cleaning validation topics highlighted in ICH Q7 [21] include:

- Cleaning validation should be performed for process steps in which contamination or material carryover poses the greatest risk to product quality.
- Cleaning validation should reflect actual patterns for equipment usage.
- Sampling should include swabbing, contact plates, rinsing, or alternate methods, as appropriate.
- A company should use validated methods that have the sensitivity to detect residues and contaminants.
- Equipment cleaning/sanitization studies should address microbiological and endotoxin contaminations, as appropriate.
- Cleaning validation should include monitoring of equipment at appropriate intervals to ensure that cleaning procedures are effective during routine production.

One of the main steps in equipment cleaning validation is selecting the best residue detection method. There are two primary types of sampling techniques widely used in cleaning validation studies and during the routine monitoring of pharmaceutical equipment and surfaces.

Each sampling method, direct surface sampling (swabbing or contact plates) and indirect (rinsing diluent or placebo), has advantages and disadvantages, which are given in Table 7.6

Table 7.6: Advantages and Disadvantages of Surface Sampling for Bioburden and Endotoxins

Test Method	Advantage	Disadvantage
Swabs	Sampling for small, difficult to reach surfaces Various selective media can be used	Much more handling of samples required (increase of potential of contamination from handling) Not very sensitive, low rate of recovery, less sensitive than contact plates Test method validation can be extensive
Contact Plates	Can purchase sterile pre-packaged and easy to use media (no transfer required like swabs) Growth occurs directly on media Can contain neutralization agents that help recover microorganisms exposed to cleaning agents Very limited handling of samples	Not very flexible for irregular surfaces Need to remove media from surface after sampling
Contact Slides	Growth occurs directly on media Can contain neutralization agents that help recover microorganisms exposed to cleaning agents Very limited handling of samples Can purchase sterile pre-packaged and easy to use media (no transfer required like swabs)	More flexible than contact plates Need to remove media from surface after sampling

Table 7.6: Advantages and Disadvantages of Surface Sampling for Bioburden and Endotoxins (continued)

Test Method	Advantage	Disadvantage
Rinse Samples	Better overall assessment due to increased coverage Excellent for sampling closed systems rinse water covers all surfaces including process pipes, valves, pumps, hoses, and tank surfaces	Potential to miss microorganisms (no physical removal) Rinse solution must be filtered on growth media (increased handling) Generally, discouraged by the FDA[18] unless justified, i.e., closed system [17]
ATP Bioluminescence	Rapid, reliable method	Validation extensive Only reliable for counts ≥ 10^4 (generally used in the food industry)

7.4.3 Bioburden and Endotoxin Interaction with Surface Materials

The evaluation of the equipment product contact surface material is the first step in the development of surface sampling microbial and endotoxin recovery studies. In most life science industries, the largest product contact surface area of material is 316 L SS. In most cases it is approximately 97% or more of the entire equipment train surface area. Therefore, it is understood that SS surface material is an ideal candidate for swab recovery studies. Also, if a detailed risk assessment is performed then a justification can be made that all other surface MOC are in such low quantities that risk of contamination of the product is very low.

As described in [95]:[19]

> *"[Based on surface assessment, product contact] surfaces can be split into several groups, for example, porous and nonporous, inert or active, rough or smooth, and hydrophobic or hydrophilic. Glass and stainless steel are examples of nonporous inert surfaces, and galvanized steel, brass, and copper are example of nonporous active surfaces. Stainless steel has been extensively studied, in part because it is the principal material of construction for equipment used in of good manufacturing practice (GMP) equipment. Microscopically, stainless steel may show grooves and crevices that can trap bacteria, but glass does not. Some bacteria have been found to be able to adhere to stainless steel surfaces after short contact times if the conditions are appropriate (i.e., adequate temperature and humidity)."*
>
> *"The porosity of a surface is a major factor affecting bacteria adherence. Highly porous surfaces facilitate adherence of bacteria. However, the adherence of bacteria depends on the number of cells—the higher the number of cells, the higher the probability those cells remain attached on surface after rinse. It was demonstrated that Gram negative bacteria adhesion could be decreased with the addition of silicone on porous material such as plastics, Teflon, and Dacron. Additionally, it was reported that rubber and plastic coupons were significantly greater bacteria growth than glass coupons, as revealed by the high population of bacteria recovered from their surfaces. Porous materials such as plastics, Teflon, Dacron, and their combination are used less often as materials of construction in GMP equipment. Reports from recovery studies indicated bacteria deposition on Teflon is greater recovery than on glass.*
>
> *"Silicone rubber has found widespread use in medical, aerospace, electrical, construction, and industrial applications. Silicone rubbers are synthetic polymers with a giant backbone of alternating silicon and oxygen atoms. The nonporous nature of silicone's surface does not allow the adhesion of bacteria. Nevertheless, studies of bacterial adhesion with laboratory strains of bacteria (i.e., type culture collection strains), many of which had been transferred thousands of times and lost their ability to adhere, first indicated that very smooth surfaces*

[18] FDA [17]: *"A disadvantage of rinse samples is that the residue or contaminant may not be soluble or may be physically occluded in the equipment."*
[19] Information from Tandon, Chhibber, and Reed [96], Williams et al. [97], Neely and Maley [98], Mafu et al. [99], Rose et al. [100], Absolom et al. [101], Egwari and Taiwo [102], Rijnaarts et al [103], Lynch [104], Xie et al. [105], Chudzik [106], as cited in [95].

> *might escape bacterial colonization. Subsequent studies with "wild" and fully adherent bacterial strains showed that smooth surfaces are colonized as easily as rough surfaces and that the physical characteristics of a surface influence bacterial adhesion to only a minor extent. This point is important to remember when selecting test microorganisms for suitability testing."* [95]

Table 7.7 shows the different interactive effects of MOC on microbials.

Table 7.7: Microorganism-Substratum Interaction for Microorganism Adherence and Survival
Adapted and used with permission from Pharmaceutical Technology [95]

Material	Surface Nature	Interaction with Microbes
Stainless Steel (SS)	Nonporous inert	Dry conditions lead to death. Some bacteria have been found to be able to adhere to the SS surfaces after short contact times if the conditions are suitable (i.e., adequate temperature and humidity).
Borosilicate glass	Porous	Bacteria and endotoxin adsorbed into porous structure making recovery difficult
Glass	Nonporous inert	Dry conditions lead to death. Bacteria are less viable than SS.
Brass, copper, galvanized steel, aluminum, aluminum alloys, and other metal alloys	Nonporous active	Toxic to bacteria due to metal ions released
Silicone rubber	Nonporous inert	Less suitable for adherence than plastic
Butyl rubber	Porous	Bacteria adsorbed into the porous structure making recovery difficult
Polyfluorocarbons	Porous inert	Bacteria adherence is more than glass but less than plastic
Polyethylene, polyurethane, polypropylene and polystyrene plastic, and rubber	Porous inert	More suitable for bacteria adherence and survival than silicone rubber, Teflon™, Dacron™, steel, brass, copper, aluminum, and metal alloys Endotoxins (LPS) will adhere and be absorbed onto surfaces making recovery difficult. Low endotoxin results post cleaning could indicate that the endotoxins have been bound to the surfaces. Need for spike and recovery study validation.

7.4.4 *General Overview of Bioburden Sampling Methods*

For bioburden recovery in cleaning validation studies, the focus is on the recovery of mesophilic aerobic microbes. For this purpose, Trypticase soy agar (TSA) with Lecithin and Tween® 80 medium incubated at 30°C–35°C is suitable. However, alternate media and incubation conditions (or D/E Neutralization media) may be required if the detection of a specified microbial species is a concern. Bacterial endotoxins are typically detected from swab and rinse samples using the Bacterial Endotoxin Test (BET) method. If swabs are used, extraction methods must be developed prior to processing the samples. Endotoxin sampling and test method validation are described in Section 7.4.

For bioburden recovery, the direct surface the method is used (swab method); after swabbing is complete (per procedure), the swab may be streaked onto an agar medium or transferred into a neutralizing diluent, the liquid diluent is vortexed for about 30 s, and the liquid sample preparation tested by pour-plate or membrane filtration method.

The incubation conditions for the recovery media vary depending on company protocols or procedures. However, in general, swab preparations are plated onto TSA with Lecithin and Tween® 80, then the plates are incubated at 30°C–35°C for 3 to 5 days. Results are reported as number of CFU per swab or area sampled. If swabs are to be transported to the testing laboratory, they need to be stored in a manner that preserves the samples collected, as well as preventing contamination.

However, in some cases two incubation temperatures may be required depending on results of the bioburden recovery studies.

7.4.5 Microbiological Direct and Indirect Sampling Method Objectives

The objective of following sections is to describe the parameters that affect cleaning validation swab recovery studies. These include:

- Test organisms spike level(s)
- Swab recovering test organisms
- Swab personnel technique
- Swab extraction
- MOC coupon
- Test method

Each of these swab recovery parameters are reviewed in detail to define best practices and highlight common mistakes to ensure successful recovery studies using a risk-based approach.

7.4.5.1 Direct Sampling – Swab Method

The USP <1072> Disinfectants and Antiseptics Methods [46] outlines considerations for an effective surface challenge test. Aside from surfaces and test species, a critical factor to consider in designing an effective test is the method used to recover test species from work surfaces. USP recommends that the:

> *"test organisms are enumerated by using swabs, surface rinse, or contact plate methods".*

Unfortunately, the use of swabs for recovery of microorganisms from surfaces has some degree of limitation mostly due to the lack of standardization of swab material, swabbing pattern, and the pressure applied to the swab during sampling. Typically, technician-to-technician variation in the surface sampling procedure may have a significant impact on the recovery and enumeration of the sampled surface. This can lead to influenced results for the initial cleanliness of the surface or the effectiveness of the cleaning procedure used. The bioburden recovery methods using swabs can be also influenced by the swab tip MOC [95]. Other key variables that may have potential direct impact on low bioburden recovery is the type of extraction fluid used, surface material sampled, method for testing bioburden, and multiple steps for handling of the samples by the laboratory technician.

Equipment swabbing should be performed by highly trained personnel and sterile swabs made from materials that do not interfere with the test method used.

Previous swab recovery studies have shown a poor correlation with the amount of microbial contamination on surfaces and the amount extracted from recovery studies [95].

> *"Many factors may contribute to this poor correlation, including differences in materials used (e.g., cotton, polyester, rayon, calcium alginate), the organisms targeted for culture, variations in surface, and differences*

> *in the personnel collecting and processing samples. Additional sources of error are the potential for non-homogenous surface deposition of microorganisms resulting in unequal or incomplete removal of microorganisms from the test surface. Based on these studies it is widely accepted that positive swab samples are indicative of high surface concentration of microbes, whereas negative swab samples do not assure that microorganisms are absent from the surface sampled."* [95]

Another factor that contributes to poor recovery is the antimicrobial effect the surface material may have on certain microorganisms. In addition, it is important to have quick turnaround for sampling and testing of samples at Time 0 and again at the end of CHT. However, the selection of commercially available swab kits from qualified vendors can aid with the standardization of the swab method. Figure 7.9 shows an example a commercial swab kit. Most commercial swab kits contain neutralization agents or dilution that are effective in neutralizing most cleaning agents and assist with the recovery of stressed microorganisms. In most bioburden studies commercially, available swab kits with neutralization dilution have been scientifically proven to have a greater recovery rate.

Figure 7.9: Commercially Available Swab Kit
Used with permission from Harmony Lab & Safety Supplies, https://harmonycr.com.

There are several types of swabs used to sample irregular hard-to-reach surfaces (e.g., impeller blades, gaskets, traps, process piping, transfer lines, and U-bends). It is understood that swab sampling should be performed using aseptic techniques, such as wearing sterile gloves, a mask, and laboratory coat to minimize the potential of cross-contamination of samples.

Indirect Sampling – Rinse Samples

Bioburden recovery for indirect surface sampling[20] is performed as follows (final rinse method): after the rinse sample is collected, it is processed via the membrane filtration technique, the filter plated onto TSA with Lecithin and Tween® 80, or Reasoner's 2A agar (R2A) and the agar plates are then incubated at 30°C–35°C for 3 to 5 days. If not properly validated, the water final rinse cycle used on the equipment surface can interfere with microbial recovery. Residual detergent or product may cause microorganism cells to lysis or inhibit recovery.

Also, rinsing and swabbing (to a certain extent) are only partially effective in removing cells from a multilayer biofilm. Companies must consider this fact when analyzing equipment cleaning data because microbial recovery methods may only provide a semiquantitative indication of the microbial contamination on equipment surfaces. Performing recovery studies is critical when determining if microbial inhibition has occurred during the cleaning process. The validation of sampling methods (swab or rinse solution), challenging test organisms, neutralization solution, and other key factors is critical in establishing acceptable bioburden limits for manufacturing equipment.

[20] This is typically used for process piping where direct method sampling is difficult to perform because of the small sample surface area and difficulty in sampling a closed system.

8 Analytical and Biological Assay Methods

Testing cleaning validation samples is critical to provide an accurate representation of the level of cleanliness of equipment both after cleaning (API or formulation; cleaning agent and bioburden as necessary) and before use (bioburden). API residue is typically tested, as it may be the high hazard component in the formulation. Any test method used must be validated for the sample being taken [21]. The primary consideration for the test method is that it is sensitive to levels lower than the cleaning limit for the analyte of interest. If the test method is not sensitive enough to test residue levels lower than the cleaning limit, the method sensitivity must be enhanced, a different test method must be employed, or the manufacturing equipment must be dedicated [17].

A specific method (e.g., HPLC) is preferred by regulatory agencies [17], but a non-specific method (e.g., TOC) can be acceptable. Bioburden testing is often conducted by compendial methods as a general test, with individual species identified as necessary.

The analytical performance characteristics, or validation parameters, should conform to those defined by ICH Q2 [107]: accuracy, precision, specificity, LOD, LOQ, linearity, range, robustness, and recovery (see Section 8.1). Definitions of the parameters are shown in Table 8.1.

8.1 Analytical Methods

8.1.1 Validation Parameters

Validation parameters are described in Table 8.1.

Table 8.1: ICH Q2 Validation Parameter Definitions [107]

Parameter	Definition
Accuracy	*"Expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found"*
Precision	*"Expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions* • *Repeatability – the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.* • *Intermediate Precision – within-laboratories variations: different days, different analysts, different equipment, etc.* • *Reproducibility – expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology)"*
Specificity/Selectivity	*"Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present."*
Detection Limit (LOD)	*"The lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value"*
Quantitation Limit (LOQ)	*"The lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy"*
Linearity	*"The ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample"*

Table 8.1: ICH Q2 Validation Parameter Definitions [107] (continued)

Parameter	Definition
Range	*"The interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity"*
Robustness	*"A measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage"*
Ruggedness	The degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, analysts, instruments, reagents lots, or days

8.1.1.1 *Accuracy*

Accuracy determines the closeness of test results to the true value across the range [107].

Samples extracted into solutions are measured and compared to standard solutions at comparable concentrations to determine accuracy. For cleaning validation samples, accuracy is measured through recovery of samples from equipment surfaces and extraction of the recovered samples into a testing solution. Accuracy should be established using a sufficient number of data points. One example is three recovery levels in triplicate, for a total of nine recoveries (see Chapter 7). Accuracy is reported as % recovery of the amount of analyte in the recovered samples measured against the amount of analyte spiked onto the sample recovery surface.

Accuracy should be determined around the ARL, which is the point around which accurate data is most essential. However, for a relatively safe product, the ARL could be relatively high. For example:

- Typical accuracy at 75%, 100%, and 125% of ARL
- If ARL > 100 µg/25 cm^2 swab, perform recovery around 100 µg/25 cm^2
- Advisable to extend linearity up to ARL to understand possible method limitations
- Advisable to extend accuracy down to method LOQ and one to three levels in between, which is where most data should be

8.1.1.2 *Precision*

Precision determines the degree of agreement among individual test results applied repeatedly to multiple samples from a homogeneous sample [107]. It is a measure of the combined variability of the sample recovery, the sample extraction, and the sample measurement. Precision may be considered at three levels: repeatability, Intermediate Precision (IP), and reproducibility. All the parameters of the recovery process (see Chapter 7) can affect the recovery of the sample. The key is to control sample parameter variability as much as possible.

During swab sample method validation, a sufficient number of recovery samples should be run to have a statistically significant RF of a somewhat variable test. The samples used for accuracy determination are also used for precision.

There is no regulatory acceptance criterion for precision for cleaning samples. Variability measured as % RSD is often used with a limit of ≤ 15% for swab samples.

8.1.1.3 Specificity

Specificity determines the ability to assess the analyte in the presence of components expected to be present in the sample matrix [107]. For cleaning samples, the expected components are the other ingredients of the formulation, potential degradants, and the cleaning agent.

Specificity is the capability of a method to separate known degradants identified during product development and previously developed selective product assays. The stability of the analyte in alkaline or acidic solutions should be reviewed to determine the risk of degradation during the cleaning process. Although the ability to detect degradants is necessary, the risk of their presence should be low. Degradants are structurally related to the analyte and removed to the same extent as the analyte. Since they are a low level to begin with, the risk of their presence in a post-cleaning sample should be minimal.

An exception to this approach is where the analyte is known to degrade in the cleaning solution. This approach can be leveraged to eliminate all analyte from the residue and then the primary degradant(s) should be monitored, its pharmacological activity determined, and appropriate cleaning limits established as necessary.

A specific method (e.g., HPLC) should be able to distinguish the specific analyte of interest from the other matrix components. A non-specific method (e.g., TOC) is not able to distinguish between the analyte of interest and other sample matrix components. Therefore, when using TOC, all measured residue has to be assumed to be the analyte of interest. HPLC and TOC generally have comparable sensitivity, so either approach is acceptable if it can measure to levels lower than the ARL. Run times are also comparable but each technology can use shortened analysis times. Table 8.2 lists the advantages and disadvantages of each approach.

Table 8.2: Advantages and Disadvantages of Specific and Non-Specific Methods

Test Method	Advantage	Disadvantage
Specific (e.g., HPLC)	• Separates analyte of interest from matrix components • Can be tested in a lower volume to increase sensitivity • Not as variable as TOC • Not limited by swab solvent • Newer instruments have shorter run times	• Longer method development timeline • Individual injections can be lengthy
Non-Specific (e.g., TOC)	• Shorter method development timeline • Newer instruments have shorter run times	• All instrument response is assumed to be the analyte of interest • Generally requires a greater dilution factor, which could limit sensitivity • Variable response, particularly at low levels (ppb range) • Swab solvent limited to aqueous

8.1.1.4 LOQ/LOD

LOQ (also known as Quantitation Limit) is the lowest amount of analyte that can be determined with acceptable accuracy and precision under the stated experimental conditions, while LOD (also known as Detection Limit) is the lowest amount of analyte that can be detected under the stated experimental conditions [107].

8.1.1.5 Linearity

Linearity is the ability of an assay to elicit a direct and proportional response to changes in analyte concentration [107].

8.1.1.6 Range

Range is the interval between the upper and lower levels of analyte that has been demonstrated to be determined with a suitable level of precision, accuracy, and linearity [107].

8.1.1.7 Robustness

Robustness is the measure of the capacity to remain unaffected by small but deliberate variations in procedural parameters, for example, changes to [107]:

- Mobile phase
- Column temperature
- Wavelength
- Injection volume
- Sample solvent

8.1.1.8 Intermediate Precision (IP)/Ruggedness/Reproducibility

Ruggedness is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different instruments, analysts (called IP), laboratories (called reproducibility), reagents lots, or days [107].

8.1.1.9 Solution Stability (Sample/Standard)

Solution stability is the measure of how long standard and samples can be retained and continue to demonstrate consistent detector response.

- Standard solution
- Sample solution after extraction
- Sample before extraction (swab)
- Dried sample on the coupon

8.1.2 Methodology

There are a variety of methods available to test cleaning validation samples. There are specific and non- specific methods. A specific method can distinguish the analyte of interest from other components of the sample matrix. A non-specific method measures the analyte of interest along with other components of the matrix that elicit a response from the test method. The method of choice is based on the most appropriate for the specific circumstances, first considering specific methods followed by non-specific methods. All test methods require validation.

8.1.2.1 Specific Test Methods

HPLC is the most commonly used specific test method for cleaning validation. The use of HPLC for testing cleaning validation samples is well established and can address all validation parameters. HPLC is a chromatographic method that passes a sample through a packed column and separates the analyte of interest from the other components of the sample. An HPLC method can separate the residue of interest from the components of the formulation as well as the detergent. A well-designed HPLC recovery study can demonstrate accuracy, precision, linearity, range, LOD, and LOQ in a single run.

Typically, HPLC methods for assay are designed to quantitate levels down to 0.1% of the API, making sensitivity well below most calculated ARLs. However, the sensitivity of HPLC is dependent on the chemical structure of the residue of interest and the detector, which can quantitate the residue.

HPLC assay methods can be lengthy, 30 to 40 min per injection, which could be an issue for quick turnaround of samples.

Use of other specific test methods such as GC, MS, or IR can be used in certain situations and are subject to the same validation parameters.

8.1.2.2 Non-Specific Test Methods

TOC is the most commonly used non-specific test method for cleaning validation. TOC analysis involves the oxidation of carbon and the detection of the resulting carbon dioxide produced. Sensitivity is down to the ppm or ppb level. A well-designed TOC recovery study can also demonstrate accuracy, precision, linearity, range, LOD, and LOQ in a single run.

In a TOC analysis, all organic carbon is detected; therefore, any residue detected must be considered the residue of interest. Residues for TOC analysis must dissolve in water. This can limit the effective linear range of the residue assay.

Use of other non-specific test methods such as pH or conductivity can be used in certain situations and require only instrument calibration.

Use of other non-specific methods (e.g. pH, conductivity) can be applied in certain situations if a strong scientific justification is provided to ensure it is appropriate and provides the necessary controls for an effective cleaning process.

8.2 Test Method Assessment for Bioburden and Endotoxin

8.2.1 Swab Recovery Method Assessment

An equipment rinse is performed using a solvent that does not interfere with recovery. The swabbing technique, although it has a special advantage over the rinse sampling method, has the major disadvantage of a low recovery of collected bioburden. The reason for low recovery is related to the swab fiber matrix, which hinders the release of microbial cells.

The recovery study results should be assessed by the QC laboratory when reviewing cleaning validation and routine microbiological data. If the recovery results are low, then an RCF should be applied to the final test results to compensate for sampling method limitations and to determine if the acceptance criteria were met.

Appendix 4 describes a standard swab test method assessment based on “A Novel Improved Bioburden Recovery Method Using Swabbing Technique” [108]. For standard bioburden recovery, after swabbing is complete typically the swab is either streaked onto an agar medium or transferred into a liquid medium, vortexed for about 30 s and then the liquid sample preparation is tested by pour-plate or membrane filtration method [37].

Appendices 4 and 5 describe the methods for collecting and extracting microorganisms from swab material. Using a commercial swab kit is recommended for this study. There are many different commercially available swabs that can be used, especially for surface sampling. Most swab wetting solutions contain an emulsification and neutralizing buffer to neutralize cleaning agents that may inhibit microbial growth. The emulsification solution extracts the microorganisms from the swab material and disperses them into the solution. This allows the recovery of any microorganisms exposed to specific cleaning agents.

It should be noted that typical recovery specifications applied to chemical cleaning validation are not applied to microbiological recovery. For bioburden limits, a 50% recovery is outstanding; usually recoveries obtained are around 5%–20%, with some companies stating only "growth" as the recovery requirement. The bioburden recovery results for some materials (porous) can even be less. However, the method described in this Guide should assist in getting recovery rates greater than 50%.

Consider that there is one factor affecting these recoveries that is normally not an issue in chemical cleaning validation: the survival of the microorganisms.

The following data is required to calculate the limits:

- Worst-case product/equipment train combination
- Lowest specification for product contamination (10^3 CFU/g aerobic bacteria, 10^2 CFU/g molds and yeasts) (solids) [46, 109]
- 10% contribution to the total count by the equipment (90% of the contamination coming from unknown sources)
- 0.1 safety factor

Per current versions of USP[21] [46] and Ph. Eur. [109], the microbial requirements for non-sterile pharmaceutical DPs are:

- Control of the total bioburden
- Elimination of USP indicator and objectionable microorganisms

Cultures usually need to be heavily diluted prior to plating; otherwise, instead of obtaining single colonies that can be counted, a so-called "lawn" forms: thousands of colonies lying over each other. Additionally, plating is the slowest method. Most microorganisms need at least 24 hours to form visible colonies. The enumeration of colonies on agar plates can be greatly facilitated by using colony counters.

To quantify the number of microorganisms in a culture, the microorganism is plated on a Petri dish with growth medium. If the microorganism cells are efficiently distributed (non-spreaders) on the plate, it is generally assumed that each microorganism cell will give rise to a single colony or CFU. The colonies are then counted and, based on the known volume of culture that was spread on the plate, the cell concentration calculated.

The primary technique in counting colonies is to count each colony dot once. One approach is to set the Petri dish on a grid background and count the colonies in each grid cell, moving in a methodical pattern through all the cells. Marking counted colonies on the back of the Petri dish can also be a helpful approach. Generally, you will need to count at least three plates; only use plates containing 30 to 300 colonies to make robust inferences. Plates with colonies that are too numerous to count or with too few colonies need to be re-plated from a new dilution. Another approach is to use a colony counter for inspection of all plates to ensure a consistent counting background.

8.2.2 Endotoxin Surface Sampling

Bacterial endotoxins are typically detected from swab and rinse samples using the LAL method. If swabs are used, extraction methods should be developed prior to processing the samples.

[21] USP <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests; USP <1111> Microbiological Attributes of Nonsterile Pharmaceutical Products [46]; Ph. Eur. 5.1.4 "Microbiological Quality of Non-Sterile Pharmaceutical Preparations and Substances for Pharmaceutical Use" [109].

Equipment swabbing needs to be performed by qualified personnel, using sterile swabs made from materials that do not interfere with the test. There are several types of swabs used to monitor flat or hard-to-reach surfaces such as the bottom of a tank, O-rings, traps, transfer lines, and U-bends, although rinse samples are much easier to take.

> *"The Bacterial Endotoxins test can be performed by the kinetic turbidimetric, kinetic chromogenic, or gel-clot test methods. However, the kinetic test methods have significant advantages over the gel-clot test."*
>
> *"A BET involves analyzing the liquid sample or sample extract using Limulus Amebocyte Lysate (LAL). LAL is a reagent made from the blood of the horseshoe crab. In the presence of bacterial endotoxins, the lysate reacts to form a clot or cause a color change depending on the technique. The test sample is compared to a standard curve made from known endotoxin concentrations. All tests are performed in at least duplicate. A positive product control and negative control are included as part of each assay."*
>
> *"It is required to demonstrate that the test sample does not interfere with the ability to detect endotoxins. This is accomplished with the positive product control (also called the spike recovery) for the kinetic test methods, and with a separate inhibition and enhancement assay for the gel-clot method."* [110]

Appendix 5 describes the recommended step-by-step approach for developing a swab and rinse recovery method for endotoxin.

8.3 Microbiological (Virus, Mycoplasma, and TSE) Studies to Support Cleaning Requirements

The challenges for cleaning validation in biopharmaceutical and biological processes are somewhat different from the traditional validation of small-molecule chemical residues. In the biopharmaceutical industry, the main issue is microbiological contamination including viral, mycoplasma, bacteria, fungi, and other biological residues, and almost all of these can be overcome by using sodium hydroxide and other alkaline solutions in the cleaning process.

Most manufacturing facilities outsource the cleaning method/cycle development activities that involve pathogenic or non-flora type organisms due to the inherent challenges and possible facility and/or laboratory contamination risks. The data generated from these studies supports the development and validation of the cleaning cycles including types of cleaning agents used, sampling methods, and other relevant process requirements. It is understood that special biological handling, precautions, and containment controls should be implemented when working with the organisms described.

Process development and optimization in upstream process includes various parts:

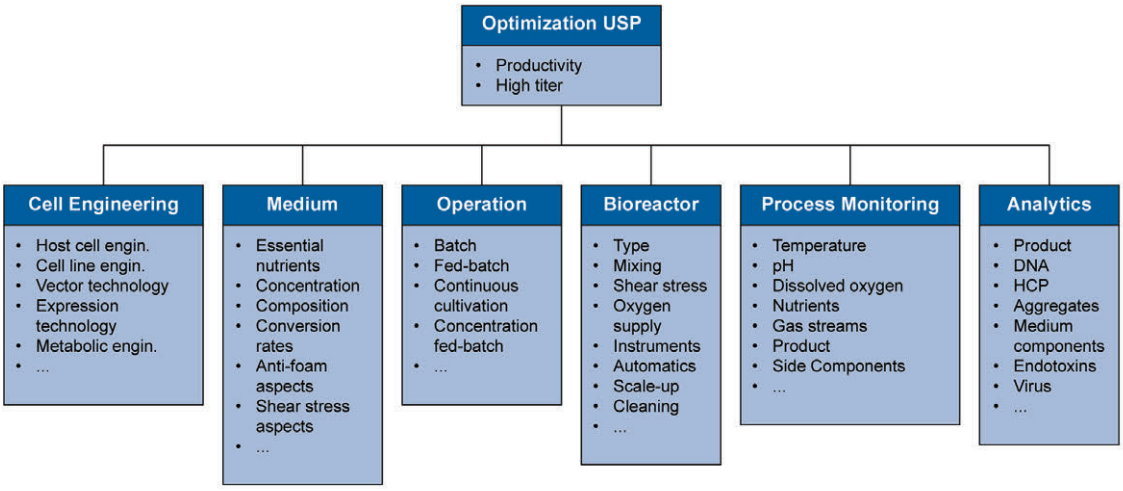
- Cell line development and engineering
- Cell clone selection
- Media and feed development
- Bioprocess development and scale up

> *"Reactor design, cell harvesting, process control and the corresponding analytics can be part of the optimization process as well…. These areas are optimized individually and focus on a robust generation of a high product titer, high productivity and defined quality….* [Figure 8.1] *schematically presents the different optimization areas and lists the most important parameters."* [111]

During the laboratory scale studies, the criterion at this stage is to perform the necessary challenges to validate the removal of specific biological agent (analytics) residues to an acceptable level from the process stream equipment. The use of cleaning agents should be controlled as well as all CPPs, and should be scalable to commercial quantities.

Following process development, process characterization, process transfer, and set up, GMP production takes place in combination with cleaning validation. Technical data from laboratory scale studies should be transferred to the process development group. When scaling up cleaning processes from laboratory scale studies (virus removal, etc.) to process development optimization, it is important to maintain some degree of control of specific cleaning parameters such as cleaning solution concentration, cleaning methodology, and other factors [111].

Figure 8.1: Optimization Areas and Parameters in Upstream Processing
Used with permission from Bioengineering [111]



Additional information can be found "A guide to planning your Cleaning Validation Study," by BioReliance [112].

> *"The selection and evaluation of model microorganisms for cleaning validation studies is a critical part of developing a removal/inactivation protocol. The selection should consider the type of equipment and raw materials used in production processes, and the model microorganisms should be known contaminants or appropriate related models. For example, bacterial and fungal species selected should be representative of environmental, human, and material source-derived microbial flora, and should include species of known antimicrobial resistance. An additional factor to consider for a model microorganism selection is its ability to grow as a high-titer stock in both standard microbiological and cell culture media, and its ease of detection in a sensitive and reliable assay…. Typical residual contaminants that can be important for cleaning validation studies include:*
>
> - *Host-cell proteins*
> - *Lipids*
> - *DNA/host-cell nucleic acid*
> - *Endotoxins*
> - *Carbohydrates*
> - *Membrane/chromatography matrix leachables*
> - *Detergents*
> - *Viruses*
> - *TSEs*
> - *Mycoplasmas, bacteria, fungi"*

9 Equipment Issues and Challenges

9.1 Design Aspects of Cleanable Process Equipment

Process equipment used for pharmaceutical manufacturing should be designed to allow cleaning of all product contact surface areas. Frequently, a process cleaning operation is a hybrid of COP and CIP; that is to say, some equipment components are removed for COP, with the remainder cleaned in place.

The following is an overview of design and fabrication aspects of equipment and processes necessary for the operation, integrity, and safety aspects of CIP integration with process equipment.

Material of Construction (MOC) – Proper selection of materials of construction is fundamental for both cleanability aspects and so as not to compromise the integrity of the equipment by the cleaning process. All equipment in contact with cleaning solutions should be made of materials such as SS, glass, or CIP-cleanable elastomers of equally corrosion-resistant construction that are validated for the intended application.

Product Contact Surface Finish – Process equipment surfaces that come in contact with the product or can be a source of external process contamination, such as from splashing or draining, are considered product contact surfaces. These surfaces should be fabricated to an appropriate specified finish (acceptable Ra value) for the process environment.

Solution Confinement – The need for containing the solution is fundamental to CIP for both operational and personnel safety reasons. Equipment must be designed to confine the solutions used for flushing, washing, and rinsing.

Equipment and Piping Support – The need for equipment and piping support is fundamental to CIP for both operational and personnel safety reasons. Equipment and piping must be provided with the necessary rigid construction to support the weight, alignment, and endure proper pitch under process and CIP loading conditions.

Automatic Welds – High-quality automatic orbital welded joints are the most suitable for all permanent connections in transfer systems constructed of SS for CIP service.

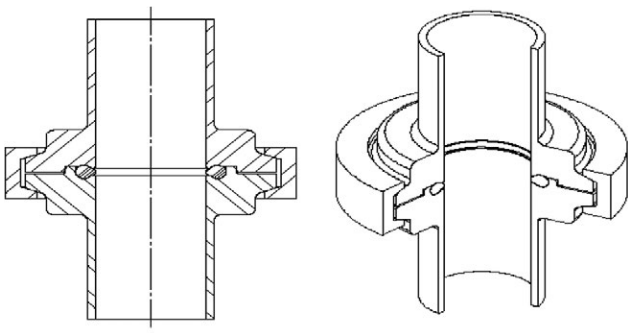
Manual Welds – High-quality manual welds meeting the following criteria are acceptable for CIP service: the weld should be sound (full penetration with no leaks, cracks, crevices, pits or protruding metal), smooth/easily cleanable, and the welded joint and associated piping are drainable.

Joint Connections – Hygienic clamp or union type joints are preferred. Other flange types for semipermanent connections are acceptable if they meet the following criteria:

- A joint and gasket assembly that maintains the alignment of the interconnecting fittings
- A gasket positioned so as to maintain a smooth/flush interior surface
- Assurance of pressure on each side of the gasket at the interior surface to avoid product buildup in crevices that might exist in joints that are otherwise “water-tight”

Threaded national pipe thread-type connections are not suitable for CIP service due to the penetration of soils into the grooves and the inability of CIP solutions to reach these areas. Compression band hose clamps are not acceptable for CIP service because of the resulting recessed groove.

Figure 9.1: Gasketed Joint with Recessed O-Ring or Seal with a Portion of the O-Ring or Seal Surface Exposed to Cleaning Solution
© 2018. 3-A Sanitary Standards, Inc. Used/Adapted with permission from 3-A Sanitary Standard for General Requirements, Standard 00-01. (https://www.techstreet.com/3a/pages/home).



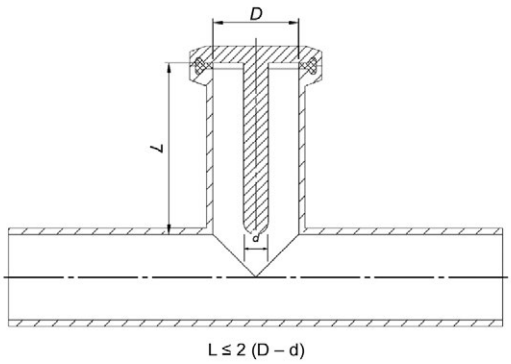
CIP-Cleanable Valves – Hygienic diaphragm or rising stem compression valves are examples of valves that are acceptable for CIP service. Valves with stems, seats, cavities, threads, and packing inaccessible by in place cleaning methods are not suitable for CIP, for example, butterfly and ball-type valves.

Corner Radius – A minimum radius of 25 mm is desirable at all corners, whether vertical or horizontal.

Mechanical Seals – In closed processing systems, the mechanical seal provides a barrier between the product contact area of rotating equipment and the external environment. For CIP and SIP applications, the seal enables the cleaning (and sterilization) of the product wetted process area with the seal in place. The mechanical seal deters microbial contamination and provides either a dust tight vapor seal, a pressure tight seal to allow pressure and/or vacuum isolation in the process, or an aseptic sealing device. The seal materials must be capable of tolerating process conditions, cleaning chemicals and temperatures, and sterilization conditions (as applicable). Seals can be of single or double-mechanical construction. They can either be dry running or be provided with isolating sterilizing media (such as clean steam or sterile water), and liquid or gas lubricant. Mechanical seals are commonly used for pumps, agitators, mixers, and other types of rotary equipment.

Dead Ends – Piping branches that are beyond the boundaries of turbulent CIP flow contact (referred to as “dead ends” or “dead legs”) should be eliminated. Branches or tees in a horizontal position and limited to a length to diameter ratio (L/D) of 2 or less are recommended. Vertical upward branches are less preferred in fluid processes as they may entrap air, preventing cleaning solution from reaching the upper portion of the fitting. Vertical downward branches may entrap particulates. Consideration should be given to the location of valves within lengths of piping, and whether or not the closure of the valve during a cleaning operation could create a difficult-to-clean dead end that does not otherwise exist when the valve is opened.

Figure 9.2: Length to Diameter Ratio (L/D) for a Tee Branch in a Process Line
© 2018. 3-A Sanitary Standards, Inc. Used/Adapted with permission from 3-A Sanitary Standard for General Requirements, Standard 00-01. (https://www.techstreet.com/3a/pages/home).



Maintenance – Deterioration of equipment occurs over time, which can adversely affect cleanability. Examples for common areas of deterioration are surface finish, mechanical seals for rotating equipment, valve components, gaskets, and O-rings. Corrective maintenance to rectify a fault and preventive maintenance ensure that the process equipment continues to maintain design aspects of CIP cleanability.

Several professional organizations with the mission of developing standards, guidelines, and practices of hygienic cleanable design have been established to address the cleanability concerns of hygienic processing. Some examples are 3-A SSI [30], EHEDG [34], and ASME BPE [32].

Appendix 7 presents a CIP cleaning process development case study.

9.2 Solid Dosage Processing

Bulk formulated solid API is processed through to a finished dose form such as capsules and tablets. Unit operations include drying, milling, extrusion, granulation, compression, and coating.

At the start of clean up after solid dosage processing, there are hold up areas that require equipment to be split down for cleaning, for example, mills, compression rollers, and compression machines.

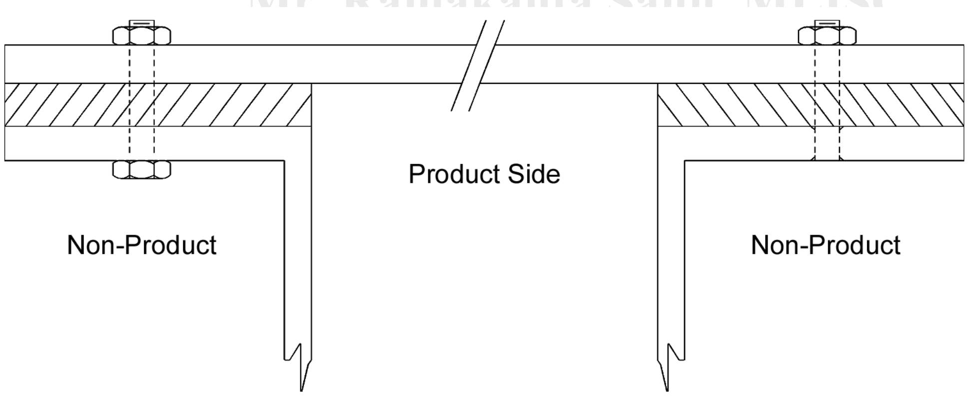
Manual cleaning between batches with a vacuum system to remove gross residues is suitable for some solid dosage processing equipment, such as tableting and capsule filling machines. These types of equipment are also available in CIP-cleanable construction such that there is complete isolation of the processing area, so that neither the powder nor the cleaning media can pass into the mechanical area.

CIP is suitable for larger bulk handling equipment such as blenders and granulation chambers. In this case, the equipment is fitted with CIP spray devices, and the CIP circuit is configured analogous to vessel washing.

Interconnecting product ducts of up to 100 mm diameter can be CIP cleaned using pumped pressurized flow at 1.5 m/s, the recommended velocity adopted by hygienic design industry standards [30, 32, 34]. Beyond a 100 mm diameter, the requisite flow rate to ensure adequate flow pressure and flooding for cleaning can become prohibitively great. Larger diameter ducts can be fitted with spray devices to provide circumferential wetting of the internal duct services. In this manner the CIP flow rate required for large diameter ducts is greatly reduced. CIP return flow of spray cleaned ducts is collected and drained at low points.

Flange-type fittings and other alternative semipermanent connections are commonly used in large diameter solids processing systems. For CIP, it is important to ensure the integrity of these joint connections (smooth surfaces that neither create a crevice nor bulge) to ensure cleaning.

Figure 9.3: Flat Sealing Surface without Radius for the Product Contact Juncture
© 2018. 3-A Sanitary Standards, Inc. Used/Adapted with permission from 3-A Sanitary Standard for General Requirements, Standard 00-01. (https://www.techstreet.com/3a/pages/home).



For multiple operation connected processes, CIP supply zones can be established by valve sequencing. An example is a spray dryer, cyclone, bag filter, and drum-out discharge loading of powder along with the interconnecting ductwork. The CIP flow rate to simultaneously provide solution coverage to all areas of the spray dryer train could be prohibitively high. Accordingly, the different functional areas might be zoned together for sequential CIP supply that covers the entire spray dryer train. Overlapping zones are used to ensure that all areas are subject to CIP. The order of sequence is also an important consideration.

Pigments, dyes, and lakes added to color tablets can be difficult to remove. By their nature, color additives are water-insoluble salts and oxides. Commonly used CIP detergents are sometimes ineffective on their own. Removal of color additives may be improved by a combination of specially formulated detergent chemistry to counteract charge attraction at the process equipment surface, along with a high flow velocity of cleaning solution.

Potent compound processing adds another layer of complexity. With the need for total manufacturing flow to be isolated, high-level containment requires taking into account closed operation cleaning operations not only for the production process, but also for product feed, discharge, and sampling. Contained split-body valves allow the isolated transfer of material. These valves are available with features permitting contained decontamination, CIP, and SIP.

See Appendix 7 – Case Study: Establishing Process Parameters for a Clean in Place Cleaning Process.

9.3 Sterile Processing

Sterile products are typically manufactured in liquid form using a variety of equipment sizes and types, such as mixing vessels, hold vessels, sterile filtration, fillers, and interconnecting piping. Sterile solid dosage forms are also available and use such equipment types as lyophilizers, fluid bed dryers, mills, sieves, and dryers.

Equipment used for sterile manufacturing must not only be designed for effective cleaning, but also for effective post-cleaning sterilization as microbial contamination is as much of a concern as chemical contamination.

For the most part, cleaning larger items of equipment such as vessels and pipelines uses CIP techniques. In these cases, as with solid dose equipment, they are fitted with CIP spray devices, and the CIP circuit is configured analogous to vessel washing. Interconnecting pipelines are usually small (75 mm or less) and can be CIP cleaned using pumped pressurized flow.

Large systems can be cleaned individually as separate zones, but the most common method is to route the cleaning fluids through each zone in a defined and repeated sequence.

For CIP systems it is important that the equipment is designed to allow for full drainage of the system after cleaning, as any remaining liquid can act as a reservoir for the growth of microbial contamination. Care should be taken that there are no adverse pipeline slopes that could prevent full system drainage. Equipment used for sterile manufacturing is often blown dry as the last part of a CIP cycle using 0.22 µm filtered dry compressed air.

Smaller items of equipment, for example, buffer vessels, are often dismantled and either hand cleaned or use COP systems such as parts washers. Very small items such as filling nozzles are typically manually cleaned and dried in drying cabinets. However, some filling machine nozzle heads can be CIP cleaned through to the filler machine buffer vessel. If filling nozzle internals are designed to be CIP cleanable, they can be incorporated as part of the CIP circuit; however the external surface of the filling nozzles may have to be manually cleaned separately.

The internal surface smoothness of the equipment is important in order to aid cleaning and prevent the adhesion and retention of microbial contamination. Appropriate surface roughness (on the order of ≤ 20 microinch Ra) should be specified.

The design of the sterile system should take into account the system sterilization that occurs after cleaning. Sterilization methods may be dry heat (170°C for 1 hour) or steam (1.06 barG/121°C for 20 min). Post sterilization, the equipment may be held under positive pressure to help maintain system integrity.

For efficiency and cost-savings, the CIP supply and SIP supply and return can share as much piping as practical with SIP and condensate paths. It is also possible to include condensate drip legs and traps as part of the CIP flow path to ensure that these areas are cleaned of potential process residue (soil) that may adversely affect sterilizing heat transfer.

Filters are an important component of sterile systems, and the filter media must be removed prior to CIP. The empty filter housing can be designed either for CIP or COP. Filters that form part of a system's sterile integrity should be tested prior to disassembly for cleaning, to ensure that the cleaning process has not adversely affected the filter's integrity prior to testing.

The use of hygienic flange-type fittings such as Tri-Clamps® or DIN fittings are often used for equipment and pipework connections. These consist of two ferrules clamped together with a gasket between them, providing a smooth, easy-to-clean internal surface without crevices.

Valve types that do not trap liquid (either product or cleaning solution post cleaning) need to be used in sterile systems; therefore, diaphragm valves are the most common type used. Proper valve orientation during installation optimizes drainability.

Pumps can be difficult to clean and sterilize. Pumps such as diaphragm pumps or peristaltic pumps that are easily cleaned and sterilized are preferred. Where possible, sterile systems should use gravity feed or pressure transfer systems.

While top or bottom-mounted mixers are often used in vessels, using magnetically coupled mixers is recommended to avoid the use of seals, which are hard to clean effectively and can be microbial hold up areas. One remaining disadvantage of magnetic coupled mixers is the difficulty to take a swab sample from the back of the mixer or to dismantle them from the vessel mount for cleaning.

Equipment should be designed with either zero or minimum dead legs and, where possible, low-profile or flangeless/flush mounted fittings used (e.g., flush mounted vision ports). Specially designed low-profile aseptic port ferrules are available. The offset distance from face of vessel sidewall to face of aseptic port ferrule is optimally ≤ 25 mm so that CIP spray flow can sheet down the vessel sidewall and cover the aseptic port ferrule without leaving a shadow area. Instrument ports should be protected with sterilizable diaphragms where possible.

The difficulty of validating manual cleaning, together with the subsequent sterilization (often using steam sterilization in autoclaves), are some of the drivers for the industry's adoption of Single-Use Technology (SUT) such as flexible tubing, capsule filters, and single-use filling needles. These are provided new for each batch or product run and are often supplied as gamma-irradiated sterile units. Once used, they are disposed of and require no post-use cleaning.

Another common cleaning design challenge that requires some consideration is the lyophilization shelves used for manufacturing multiple products. Even though the lyophilization shelves are considered indirect product contact areas, there has been considerable debate within the industry concerning cleaning requirements for these surfaces. Effectiveness of CIP is confirmed initially during system qualification by spraying the chamber with a detectable marker material (e.g., Fluorescein) and confirming removal by UV light with an appropriate requalification period. No routine specific or non-specific testing is normally required as the shelf surface is not direct product contact. A risk-based approach should be developed to identify if additional controls are needed, especially when the equipment is allocated for high hazard actives.

9.4 Liquids, Creams, and Ointments

Processing equipment used for liquids, creams, and ointments is characterized by the need to form, blend, and pump viscous emulsions consisting of the API plus additives such as oil, water, waxes, lubricants, emulsifiers, stabilizers, thickening agents, and coloring. Unit operations include powder/liquid dispersers, homogenizers, rotor-stator mixers, double planetary mixer vessels, high-shear mixers, and positive displacement pumps. Recirculation and transfer piping also require cleaning.

Vessels used in these operations are built to the same hygienic cleanable design standards as those used for similar pharmaceutical operations. For example, products that are susceptible to microbial contamination require equipment with similar design features to the ones used to manufacture sterile products.

Mixing shafts, impellers, and baffles require rounded corners and edges to prevent entrapment of soil and to permit cleaning solution flow across the surface. Welded construction to the fullest extent possible is preferred. Exposed threads should not be used as a method of component attachment, to avoid soil entrapment not accessible by cleaning solution. When threads are used, isolating O-rings are located between the threads and product contact area. Cap nuts, if used, should have rounded heads, not square edges, and be sealed with isolating O-rings.

Hygienic mechanical seals isolate the mixer shaft at the point of entry to the vessel. If a steady bearing is required on the vessel bottom to support the mixer shaft, it should be of design and tolerance to permit cleaning solution flow across surfaces.

Pitched-blade “marine propeller” type mixers with rounded blade edges can be cleaned by slow rotation in the CIP coverage provided by spray devices located above. Mixers having a flat plate and axial blades, such as a Rushton turbine, require spray-device coverage from both above and below the flat plate surface.

9.5 API Processing

The typical acid, base, and surfactant chemistries used for cleaning biological soils may not be applicable to API process residues. These detergents target proteins, lipids, and carbohydrates through hydrolysis and degradation. However, solubility and degradation of compounds used in API processing depend on the specific chemical properties of the residue material. In some case, volatile solvents are needed. It is also possible, depending on the cleaning chemistry adopted, to recirculate solvent first, followed by acid and base detergents for bioburden and surface mineral buildup removal.

Depending on the nature of the product, chemical synthesis and processing of API materials can require specialized corrosion-resistant equipment. Where SS or other corrosion-resistant alloys are suitable for the process environment, equipment is fabricated following hygienic cleanable design standards and practices [30, 32, 34]. In some cases, product contact surfaces of processing equipment are lined and/or coated with corrosion-resistant materials such as borosilicate glass and PTFE. Incorporating corrosion-resistant linings and coatings requires increased distance and radius dimensions beyond that generally accepted for hygienic cleanable design.

High pressure flange-type fittings typical in API processes are suitable for CIP cleaning if the gaskets are centered flush with the internal diameter and neither creates a crevice nor bulge.

Large diameter piping such as product addition chutes, transfer chutes, and overhead condenser piping up to 100 mm diameter can be CIP cleaned using pumped pressurized flow. At 100 mm diameter and beyond, the piping can be fitted with spray devices to provide circumferential wetting of the internal surfaces. In this manner the necessary CIP flow rate can be greatly reduced.

Traditional cleaning of API reactor vent condensers is accomplished by solvent boil out and reflux. This method uses a significant volume of solvent and extended time, and the result may not be total cleaning. Special accommodation is often possible in the design of vent condenser heat exchangers to achieve effective CIP cleaning by either recirculation and/or spray-device coverage.

It is sometimes necessary to use CIP cleaning for major equipment such as vessels and incorporate COP for removable non-CIP valves and other components.

9.6 Biotechnology Equipment

Biotechnology products can be defined as recombinant proteins or therapeutic peptides manufactured using fermentation or bioreactor-type processes. Biotechnology equipment can be considered as three distinct processes:

- Upstream processes consisting of bioreactor chains
- Downstream/purification systems that usually consist of discrete unit operations for each part of the purification process
- Fill and finish operations

Biotechnology equipment is similar to that of sterile products, with the following differences:

- A sterilization process may precede cleaning due to pathogenic organism characteristics or risk of survival post-processing, or the need to treat waste product residues prior to disposal.
- If a preceding sterilization process has not been used, the waste cleaning solution may contain live organisms; thus the used cleaning solution may be sent to a "kill" system before release into the main waste system.
- Cleaning validation may be simplified for upstream systems if it can be shown that the cleaning process causes denaturation or inactivation of the active proteins, and that these inactivated or denatured fragments do not cause pharmacological effects, or if residues can be demonstrated to be removed in subsequent purification stages [21].

Upstream processes typically consist of bioreactors, media preparation vessels, and post bioreactor equipment such as centrifuges, filtration systems, and homogenizers. These can be cleaned using conventional CIP systems.

As with sterile product systems, it is important that dead legs in vessels and instrument tees are minimized and mounted vertically or with a downward slope to ensure full drainability. All pipelines and interconnections should be fully drainable and have a downward slope toward the drain point. Due to the complexity of the processing equipment, the CIP is often split into cleaning zones and sequentially operated CIP routes. A mixture of single-pass rinses and recirculated cleaning washes are used.

CIP supply and SIP supply pipelines usually share as much piping as practical, likewise CIP and steam condensate pipelines. As both CIP and condensate lines usually share common low point drain points it is typical to combine these. Condensate traps should include course filtration to prevent blockage of the trap by process residue.

Downstream operations consist of single unit operations such as chromatography and ultrafiltration systems. There are many considerations related to cleaning chromatography resin columns and ultrafiltration membranes. When designing the processing steps for the chromatography resins and membranes, the process development team may develop a cleaning process based on purpose of use and vendor recommendations. Specialized assessments of cleaning effectiveness are used for cleaning validation of chromatography resins. If fixed vessels are used, they are usually fitted with spray balls and cleaned using fixed or mobile CIP systems. Mobile vessels are typically cleaned by moving them to a fixed location CIP system cleaning station.

Fill/finish operations are very similar to, and often identical to those used for sterile solutions, and are cleaned in the same way.

As with sterile product systems, SUT is replacing buffer and media vessels, interconnecting pipelines, filters, as well as complete bioreactors up to the 2000 L size, and complete SUT final filling systems.

9.7 Clinical and Investigational Medicinal Products (IMPs)

For clinical or IMPs, cleaning verification is acceptable. In such cases, there should be sufficient data from the verification process to support a conclusion that the equipment is clean and available for further use. The equipment design, inspection, test methods, and acceptance limits to be used after cleaning should reflect the nature of cross-contamination risks [113, 114]. However, when conducting cleaning validation on clinical and IMP equipment, the principles and recommendations presented in this ISPE Guide apply.

Development of cleaning procedures may occur during the development phase so that when the product is ready for commercial production, cleaning procedures are established for the validation campaign.

9.8 Packaging Equipment

Packaging equipment may directly affect the quality attributes of the product or may have surfaces with direct product contact. Examples include tablet fillers, liquid fillers, and blister machines. Some product contact surfaces such as feed brushes on tablet blister lines cannot be easily cleaned and verified, hence requiring them to be dedicated to each product.

Cleaning considerations for primary packaging equipment are no different from other equipment where product is in contact with the surfaces. Product contact surfaces should be cleaned and validated using the same principles applied to other product contact or critical surfaces. The procedures to clean the equipment may include manual steps that need to be defined and validated. The extent of cleaning or the use of dedicated parts in the equipment are evaluated via a risk assessment to determine risks for cross-contamination. Dedicated lines (single product equipment) may require verification of cleaning steps and the removal of product initially manufactured after the event.

9.9 Dedicated Equipment

To reduce the risk of cross-contamination from other products, equipment may be dedicated to a single product or similar group of products. Cleaning validation should address visual cleanliness, cleaning agent removal, potential microbial contamination, manufacturing campaign length, and hold times (DHT, CHT). There is also the expectation that API removal is consistent. Therefore, API cleaning data might be requested. The cleaning procedure should avoid the risk of contamination from product buildup or degraded product. Refer to Section 6.1.6.

Once the cleaning process is validated, cleanliness effectiveness is monitored at appropriate intervals [20]. All dedicated equipment needs to be clearly identified and any change in the dedication status evaluated via change control.

9.10 Single-Use Technology Equipment

The application of SUT equipment enhances speed to market and drives innovation. The use of SUT represents a trend in industry when the market desires a fast setup of smaller batches for clinical and launch purposes. In principle, the disposable elements used as part of SUT may not require cleaning given their single-use application. However in this case, the vendor specification should state the precise cleanliness requirements of the as-received equipment.

Current supplier offerings for single-use systems include most equipment needed for a manufacturing process (e.g., containers, mixers, bioreactors, centrifuges, chromatography columns, filters, pumps, isolators, and fillers). Single-use connectors, sensors, sampling systems, and tube welders support the operational needs.

Large-scale processes may incorporate a hybrid approach of some SUT components along with those that are cleaned between use by either CIP or COP.

10 Manufacturing Operational Approaches and Impact to Cleaning Practices and Requirements

The operational landscape is extensive when we consider the different types of technologies, designs, and approaches that then can be used to manufacture regulated products:

- Facility layouts (e.g., level of segregation between different operations, material and equipment flows, and environmental conditions)
- Manufacturing process and platforms (e.g., batch processing, continuous operations, campaigns, hold times, and containment)
- Operational philosophy (e.g., single product versus multiproduct, line clearance approach, product changeovers, and just-in-time operations)

All these options may influence the cleaning strategy. This chapter reviews some of the most common scenarios and offers points to consider when determining cleaning requirements.

10.1 Facility Layouts and Segregation

Facility layouts are usually designed to fit the process and operational requirements. However, facilities may be expanded or repurposed to manufacture different products, and therefore, it is common to adapt processes and systems to existing facilities. Facility layouts influence how items are moved in or out of operational areas (flows). Facility flows impact the movement of material, product, samples, personnel, equipment, and waste. Segregation concepts are applied to these flows to avoid any negative impact to product quality or personnel safety. For example, crossing product flow and waste flow throughout the facility is usually avoided to ensure no cross-contamination happens to product during operation.

Controls are used to mitigate risks from cross-contamination. The basic cleaning requirements for product contact surfaces will not change; however levels of bioburden in the environment, facility surface contamination levels, and distance between operational areas may influence the approach to some aspects of the cleaning program (e.g., level of gowning, microbial monitoring programs, sanitization and disinfection approaches, sample storage locations, surface wipe downs, and containment needs).

Additional physical segregation measures can be used to control the risks of cross-contamination. For example, the use of a central washroom to clean equipment instead of cleaning in the same process rooms can mitigate the exposure of cleaned equipment to certain environmental contaminants. A central washroom should consider clean/dirty equipment storage and segregation of initial rinsed equipment from final rinsed/dried equipment.[22] In processes prone to contamination by adventitious agents and viruses, physical barriers are designed to segregate pre-viral filtration streams from post-viral filtration streams. Another segregation scheme is time segregation, that is, not executing certain operations while other operations are in process.

[22] From a cross contamination view point it may be preferred to wash the equipment as much as possible in the room. A central wash room may have opportunities to increase the risk of cross contamination if not designed or operated properly. A risk assessment should be considered to determine the best control measures to achieve proper segregation.

10.2 Manufacturing Process and Platforms

10.2.1 Manufacturing Platforms

Manufacturing technologies are always evolving to ensure quality, increase flexibility, and improve costs. Platform models provide a fixed set of equipment and unit operations to maximize the flexibility when manufacturing similar processes (e.g., small-molecule formulation, tableting operations, and monoclonal antibodies). Platform processes also facilitate technology transfers between manufacturing sites and provide opportunities to gain efficiency in execution.

When designing a cleaning program for platform processes, process hold volumes, process durations, and other process parameters can be defined for the platform. This standardization can allow justification of a simplified cleaning program with defined parameters that do not change as products change (e.g., sampling locations, sampling frequency, and visual inspection procedures). The types of soilants or worst-case product characteristics define the remaining cleaning parameters.

10.2.2 Continuous Manufacturing

Continuous manufacturing offers another opportunity to improve quality, productivity, and efficiencies over batch processes. This technology increases the use of sensors and in-line analytics to measure and monitor CPPs. Additional aspects to consider include:

- Introduction of cleaning cycles as part of continuous processing
- Justification of sampling approach (e.g., sampling type, frequency, locations)
- Justification of worst-case soilants when process stream characteristics change
- Justification for cleaning versus use of disposables for components and sensors
- Approach to address biofilms and scaling
- Impact of dismantling equipment to conduct cleaning
- Definition of CHTs and DHTs
- Justification of cleaning frequency

10.3 Equipment Selection

The selection of equipment may influence the cleaning approach. Common requirements to support robust cleaning processes include:

- MOC – compatibility with cleaning agents
- Surface finish – to prevent hard-to-clean surfaces
- Prevention of dead legs
- Drainability – to prevent water stagnation after cleaning
- Processing velocity and volumes – to promote turbulent flows and cleaning agent coverage
- Selection of valves – to ensure they have the right MOC, compatible product contact gaskets, are cleanable and drain properly

Refer to Chapter 9 for additional equipment design considerations.

10.4 Non-Product and Indirect Product Contact Surfaces

The main reason for maintaining clean equipment is to prevent cross-contamination and, therefore, the adulteration of products. Non-product contact surfaces may represent a risk of contamination for other product surfaces, and should be addressed as part of the overall cleaning strategy for the system or facility. For example, surfaces that remain dirty may accumulate dust or residues from previous batches and with time, represent a risk of contamination to other surfaces. Non-product contact surfaces should be VC. The frequency and extent of cleaning non-product contact surfaces depends on the equipment type, frequency of use, and the assessment of risks for cross-contamination.

Examples of non-product contact surfaces are floors, walls, desks, and ceilings within processing suites.

Another surface area that can impact the overall control strategy for risk mitigating contamination carryover events is indirect product contact surfaces. These areas are considered a part of equipment that is shared among multiple products yet are not intentionally subjected to the API processing pathway.

Examples of indirect product contact surfaces are:

- Interstitial spaces within tablet presses and encapsulators
- Fluid bed dryer filters housing
- Tray drying oven cavities
- Lyophilization shelves

These two surface areas (non-product contact and indirect product contact surfaces) have procedural cleaning and monitoring regimens that are risk-based driven; however, these are not considered within the scope of cleaning validation.

The boundaries of cleaning validation should focus its foundation and analytical data gathering for process controls around the product contact surfaces.

10.5 Operational Philosophy

Operational philosophy should be considered when implementing a cleaning program. For example, a facility may want to minimize the use of shared equipment for products that are hard to clean to increase operational efficiency. Dedicated equipment or dedicated change parts may be used for these types of products. This decision simplifies the cleaning requirements and increases manufacturing productivity.

Alternatively, the same facility may prefer maximum flexibility by not dedicating equipment to a particular product. In this case, the cleaning program needs the appropriate rigor to ensure that clean equipment is used, irrespective of product type.

Manufacturing Campaigns

It is common to adopt campaigns as a means to increase productivity in operations by minimizing changeover and cleaning events. When justifying campaign operations, consider qualifying a baseline process (e.g., one batch per campaign), and then evaluating extending the campaign to additional batches in a stepwise fashion. This approach allows for gathering the necessary data needed to justify longer processing times. A worst-case condition takes into consideration the residue attributes, campaign durations, campaign frequency, time between regular cleaning events, and interruption times between batches. Product quality and residues carried into subsequent batches should be considered as part of the cleaning validation approach.

11 Change Control

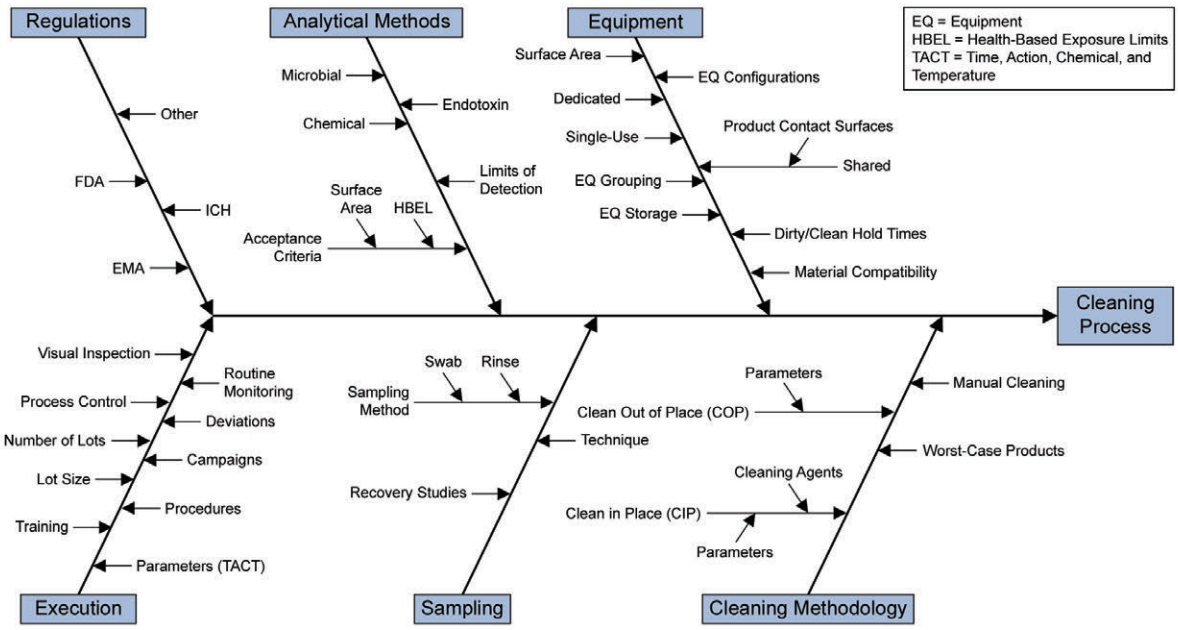
Once a validated cleaning program is in place, changes need to be managed to ensure that the cleaning process remains in a state of control. Change management is the systematic approach to evaluate, approve, and implement changes. It includes the oversight of all changes potentially affecting CPPs and/or cleaning effectiveness throughout the cleaning process lifecycle.

The company's quality system should have in place the necessary procedures to define and establish the change management system. Using QRM principles, the change management system categorizes changes according to the risk to the validated cleaning process. These risks range from high impact to low or no impact on the cleaning process. This section provides principles and guidance regarding actions to take when changing validated cleaning processes. Given the variety of industries, products, and manufacturing processes, the established company's quality system takes precedence over the principles provided in this section.

11.1 Elements of Validated Cleaning Processes

A validated cleaning process depends on multiple aspects to ensure consistency and reproducibility, with the most critical aspects monitored. Changes to these elements are controlled via change management. The fishbone diagram in Figure 11.1 illustrates the control elements of a validated cleaning process.

Figure 11.1: Elements of a Validated Cleaning Process



Modifications to a cleaning process require an impact assessment and may initiate change management. Changes to cleaning methods, introduction of new products, changes to manufacturing processes (e.g., parameters, lot size), critical parameters, equipment configurations or surface area, or analytical methods are potentially high impact changes that trigger change management. All changes to cleaning are evaluated for impact by SMEs and cleaning personnel.

The following sections provide examples of possible actions to take when certain changes are executed. The changes illustrated in Tables 11.1 to 11.5 show that there is a relationship between the impact of the change (low, medium, high) and actions necessary to keep the cleaning process in control. Typically, the greater the change impact to a cleaning process, the more actions needed to bring it back to a validated state. The tables do not cover all possible examples or conditions.

The change management system ensures that every relevant change is reviewed and assessed for impact to the cleaning validation status. Thus, a determination can be made if revalidation or further actions are required.

11.2 Examples of Changes with Corresponding Actions

Tables 11.1 to 11.5 contain examples for illustrative purposes only. The tables do not present all possible changes; however, they provide scenarios for change management actions based on the relative risks and impact of the change to the ability to clean. It is not possible to define the appropriate actions for all cases because this depends on the risk profile for each facility, the degree of knowledge of the cleaning process, and the details submitted to the regulatory dossier for the product.

11.2.1 Technical Systems and Equipment Design

Technical systems include all equipment, facilities, utilities, and unit operations used to execute the cleaning process. Systems have a combination of design features (MOC, gaskets, hold volume, etc.), instruments (temperature, flow, pressure, conductivity, etc.), and procedures that make them unique. Changes to technical systems require assessment for impact. See Table 11.1.

Other aspects to consider when evaluating changes to equipment and technical systems are:

- Coverage of cleaning agents in equipment and pipes (riboflavin tests, flooding cycles)
- The use of spray balls (pressure, angle of operation, flow rates)
- System hold volumes (pipes, tanks, valves)
- Level of dryness after cleaning cycles (dried for storage, drained, no stagnant liquids)
- Maintenance procedures (post maintenance cleaning cycles, like-for-like replacements, gaskets and other product contact components)

Table 11.1: Example Change Evaluation Assessment

Cleaning Area	Changes to…	May Impact…	Impact to Validated Status			Possible Actions 1 = Documentation Update 2 = Verification 3 = Validation 4 = Regulatory Submission			
			L	M	H	1	2	3	4
Technical Systems (Facilities, Equipment, Systems)	New Material of Construction	Compatibility, Cleanability			x	x	x		x
	Extending Elastomer Changeover Frequency	Cleaning Effectiveness		x		x		x	
	Maximum Area to be Cleaned	Cleaning Effectiveness		x		x		x	
	Cleaning Parameters within Design Space	Cleaning Effectiveness	x			x	x		
	Cleaning Parameter beyond Design Space	Cleaning Effectiveness			x	x		x	x
	Increase in Level of Automation	Cleaning Effectiveness		x		x		x	
	Manual Cleaning to Automated Cleaning	Cleaning Effectiveness		x		x		x	
Impact to validated status: L = Low Impact, M = Medium Impact, H = High Impact									
Possible Actions: 1. Documentation Update means revising existing documents, plans, or procedures to add new information or conditions. 2. Verification means verifying aspects of a cleaning procedure or extending a parameter without executing a full cleaning revalidation. 3. Validation means executing a full cleaning validation. 4. Regulatory Submission means submitting changes to regulatory agencies for review and/or approval.									

11.2.2 Cleaning Methods

The cleaning method affects the degree of action on the surface to be cleaned. Changes to cleaning methods (manual brush, wipe downs, chemical cleaning, automated cleaning, etc.) can have an effect on the cleaning effectiveness and conditions. When evaluating cleaning method changes, it is useful to understand what constitutes worst-case conditions and if the proposed changes will still operate within those conditions. If a change introduces parameters outside the known design space, an impact assessment is required to evaluate the potential impact to the cleaning process. Actions to maintain the cleaning process in a validated state range between documenting the change to full revalidation of the new parameters. See Table 11.2.

Table 11.2: Example Cleaning Methods Assessment

Cleaning Area	Changes to…	May Impact…	Impact to Validated Status			Possible Actions 1 = Documentation Update 2 = Verification 3 = Validation 4 = Regulatory Submission			
			L	M	H	1	2	3	4
Cleaning Methodologies	Critical Cleaning Parameters	Cleaning Effectiveness			x	x		x	x
	Non-Critical Parameters	Cleaning Consistency	x			x	x		
	Cleaning Solutions	Cleaning Effectiveness		x		x		x	x
	Cleaning Procedures	Cleaning Effectiveness		x		x	x		
	Worst-Case products	Cleaning Limits			x	x		x	x
	Dirty Hold Time Change	Cleaning Effectiveness			x	x		x	x
	Clean Hold Time Change	Product Quality			x	x		x	x
	Increasing Campaign Length	Worst-Case Conditions			x	x		x	x

Impact to validated status: L = Low Impact, M = Medium Impact, H = High Impact

Possible Actions:
1. Documentation Update means revising existing documents, plans, or procedures to add new information or conditions.
2. Verification means verifying aspects of a cleaning procedure or extending a parameter without executing a full cleaning revalidation.
3. Validation means executing a full cleaning validation.
4. Regulatory Submission means submitting changes to regulatory agencies for review and/or approval.

11.2.3 Sampling

Changes to sampling methods (swabs, wipes, instrument scanning, rinse samples) are assessed for impact to recovery studies and cleaning processes. Sampling locations are justified prior to validation; therefore, changes to sampling locations are assessed for impact to the cleaning process. See Table 11.3.

Table 11.3: Example Sampling Changes Assessment

Cleaning Area	Changes to…	May Impact…	Impact to Validated Status			Possible Actions 1 = Documentation Update 2 = Verification 3 = Validation 4 = Regulatory Submission			
			L	M	H	1	2	3	4
Sampling	Sampling Location	Worst-Case Assumptions		x		x			
	Sample Recovery Technique (Rinse versus Swab, etc.)	% Recovery		x		x	x		
	Storage	Sample Stability			x	x			
	Swab Area	% Recovery, Location,		x		x	x		
	Coupon Material of Construction	% Recovery		x		x	x		
	Residue Spike Level	% Recovery		x		x			
	Number of Spike Levels and Replicates	% Recovery, CV Program			x	x	x		
	Recovery Factor Determination	% Recovery, CV Program			x	x	x		
	Type of Swab	% Recovery, CV Program			x	x	x		
	Number of Swabs	% Recovery, CV Program			x	x			
	Swab Solvent	% Recovery, CV Program			x	x	x		
	Sample Container	% Recovery, Test method		x		x			
	Extraction Solvent	% Recovery			x	x	x		
	Extraction Technique	% Recovery			x	x	x		
	Extraction Time	% Recovery			x	x	x		
	Test Method	Data		x		x		x*	
	Sampling Method	% Recovery			x	x	x		

Impact to validated status: L = Low Impact, M = Medium Impact, H = High Impact
CV = Cleaning Validation

Possible Actions:
1. Documentation Update means revising existing documents, plans, or procedures to add new information or conditions.
2. Verification means verifying aspects of a cleaning procedure or extending a parameter without executing a full cleaning revalidation. Validate the method and show equivalence to the previous sampling parameter.
3. Validation means executing a full cleaning validation.
4. Regulatory Submission means submitting changes to regulatory agencies for review and/or approval.

*Method validation and equivalence determination to assess impact to cleaning process

11.2.4 Analytical Methods and Testing

Analytical methods are validated to ensure accuracy, precision, specificity (as applicable), robustness, and linearity. Changes to validated analytical methods require appropriate assessments for impact to the analytical controls. See Table 11.4.

Table 11.4: Example Methods Changes Assessment

Cleaning Area	Changes to…	May Impact…	Impact to Validated Status			Possible Actions 1 = Documentation Update 2 = Verification 3 = Validation 4 = Regulatory Submission			
			L	M	H	1	2	3	4
Testing Methods	Chemical Testing Methods	LOD or LOQ			x	x			
	Worst-Case Product Requiring New Analytical Methods	LOD, Acceptance Criteria			x	x		x	
	Chemical Testing Going from a Titration Method or TLC to HPLC	LOD or LOQ			x	x		x	x
	Microbial Methods Going from Traditional Microbiology to Rapid Microbiology Method	Microbial Test Results			x	x		x	x
	Visual Inspection Method and Training Personnel (using spiked coupons)	Ability to Detect Residues			x	x		x	
Impact to validated status: L = Low Impact, M = Medium Impact, H = High Impact LOD = Limit of Detection, LOQ = Limit of Quantitation									
Possible Actions: 1. Documentation Update means revising existing documents, plans, or procedures to add new information or conditions. 2. Verification means verifying aspects of a cleaning procedure or extending a parameter without executing a full cleaning revalidation. 3. Validate the method and show equivalence to the previous method parameter. 4. Regulatory Submission means submitting changes to regulatory agencies for review and/or approval.									

11.2.5 Residue Limits, Acceptance Criteria, and Specifications

Residue limits are established to ensure residues or contaminants from previous products do not impact the quality attributes of the next products manufactured. As a minimum, cleaning processes should render surfaces visually cleaned. Additional criteria are established for active ingredients and cleaning agents. Changes to these limits require assessment for impact to the cleaning validation. See Table 11.5. See also Appendix 8 for a case study to introduce a new product into a multiproduct facility.

Table 11.5: Example Residue Limits Assessment

Cleaning Area	Changes to…	May Impact…	Impact to Validated Status			Possible Actions 1 = Documentation Update 2 = Verification 3 = Validation 4 = Regulatory Submission			
			L	M	H	1	2	3	4
Acceptance Criteria	New Product Introduction not Representing a Worst Case for Cleaning	Worst-Case Calculation	x			x	x		
	New Product Introduction Representing a Worst Case for Cleaning	Cleaning Effectiveness, Cleaning Limits			x	x		x	x
	New Regulatory Requirement Beyond Current Cleaning Program	Cleaning Effectiveness			x	x		x	
Impact to validated status: L = Low Impact, M = Medium Impact, H = High Impact									
Possible Actions: 1. Documentation Update means revising existing documents, plans, or procedures to add new information or conditions. 2. Verification means verifying aspects of a cleaning procedure or extending a parameter without executing a full cleaning revalidation. 3. Validation means executing a full cleaning validation. 4. Regulatory Submission means submitting changes to regulatory agencies for review and/or approval.									

12 Appendix 1 – Example: Swab Recovery Execution Studies

This appendix presents swab RF determination examples (shown in Tables 12.1 and 12.2) using an acceptance range for the RF determination of 70%-110% of the spiked amount, with ≤ 15% RSD. Detailed discussion is found in Section 7.1. Additional information is contained in Chapter 6.

Table 12.1: Recovery Execution Examples

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Example 1				**Example 2**			
Residue	**API** (ARL = 100 µg/25 cm² sample)			**Residue**	**API** (ARL = 100 µg/25 cm² sample)		
Coupon Type	Stainless Steel			**Coupon Type**	Stainless Steel		
Spike Level: 125 µg/sample	Analyzed (µg/sample)	% Recovery	% RSD	**Spike Level: 125 (µg/sample)**	Analyzed (µg/sample)	% Recovery	% RSD
Coupon 1	114.0	91		**Coupon 1**	114.0	91	
Coupon 2	113.4	91		**Coupon 2**	113.4	91	
Coupon 3	112.3	90		**Coupon 3**	112.3	90	
Mean of 3 Coupons	113.2	91	0.5	**Mean of 3 Coupons**	113.2	91	0.6
Spike Level: 100 µg/sample	Analyzed (µg/sample)	% Recovery	% RSD	**Spike Level: 100 µg/sample**	Analyzed (µg/sample)	% Recovery	% RSD
Coupon 1	90.3	90		**Coupon 1**	90.3	90	
Coupon 2	92.5	93		**Coupon 2**	92.5	93	
Coupon 3	87.6	88		**Coupon 3**	87.6	88	
Mean of 3 Coupons	90.1	90	2.0	**Mean of 3 Coupons**	90.1	90	2.8
Spike Level: 50 µg/sample	Analyzed (µg/sample)	% Recovery	% RSD	**Spike Level: 50 µg/sample**	Analyzed (µg/sample)	% Recovery	% RSD
Coupon 1	46.4	93		**Coupon 1**	46.4	93	
Coupon 2	44.8	90		**Coupon 2**	44.8	90	
Coupon 3	44.5	89		**Coupon 3**	44.5	89	
Mean of 3 Coupons	45.2	90	1.7	**Mean of 3 Coupons**	45.2	90	2.3
Spike Level: 5 µg/sample	Analyzed (µg/sample)	% Recovery	% RSD	**Spike Level: 5 µg/sample**	Analyzed (µg/sample)	% Recovery	% RSD
Coupon 1	4.0	80		**Coupon 1**	3.0	60	
Coupon 2	4.2	84		**Coupon 2**	3.2	64	
Coupon 3	4.1	82		**Coupon 3**	3.1	62	
Mean of 3 Coupons	4.1	82	1.7	**Mean of 3 Coupons**	3.1	62	2.3
		% Recovery	% RSD			% Recovery	% RSD
Average % Recovery (n=12)		89	4.5	**Average % Recovery (n=12)**		89	1.5
In Example 1, all recoveries are >70% and the overall % RSD is < 15%. The RF is the average of all recovery data.				In Example 2, the recoveries at 5 µg/sample are below the > 70% recovery level and an investigation cannot improve the recovery (see below).			

In Example 2, the recovery uses only the 9 samples, which are averaged. Recoveries could be attempted at levels between the 50 µg/sample and the 5 µg/sample to identify the limit of accurate recovery. This is probably not value added, since the low level is 20 times lower than the ARL and accuracy is more critical near the ARL. It is good to understand that data around a 5 µg/sample is not as accurate; however, this limited accuracy is not going to impact whether samples pass or fail the ARL cleaning limit.

The same strategy would be used if the % RSD exceeded 15%.

Example 2 is also a good example of why not to use the single lowest recovery as the RF. In this example the lowest RF is 60%. If this was used to qualify personnel for swabbing, then personnel would likely recover material at 90% and thus not qualify since their RF is 30% higher than the established RF of 60%.

Table 12.2: Additional Recovery Execution Examples

Example 3					**Example 4**			
Residue	API				**Residue**	API		
Coupon Type	Stainless Steel				**Coupon Type**	Anodized Aluminum		
Spike Level: 125 µg/sample	Analyzed (µg/sample)	% Recovery	% RSD		**Spike Level: 125 (µg/sample)**	Analyzed (µg/sample)	% Recovery	% RSD
Coupon 1	137.0	110			**Coupon 1**	75.0	60	
Coupon 2	135.5	108			**Coupon 2**	93.8	75	
Coupon 3	139.3	111			**Coupon 3**	62.5	50	
Mean of 3 Coupons	136.6	110	0.7		**Mean of 3 Coupons**	77.1	62	16.7
Spike Level: 100 µg/sample	Analyzed (µg/sample)	% Recovery	% RSD		**Spike Level: 100 µg/sample**	Analyzed (µg/sample)	% Recovery	% RSD
Coupon 1	90.3	90			**Coupon 1**	65.1	65	
Coupon 2	92.5	93			**Coupon 2**	50.0	50	
Coupon 3	87.6	88			**Coupon 3**	44.9	45	
Mean of 3 Coupons	90.1	90	2.8		**Mean of 3 Coupons**	53.3	53	15.9
Spike Level: 50 µg/sample	Analyzed (µg/sample)	% Recovery	% RSD		**Spike Level: 50 µg/sample**	Analyzed (µg/sample)	% Recovery	% RSD
Coupon 1	46.4	93			**Coupon 1**	32.5	65	
Coupon 2	44.8	90			**Coupon 2**	27.6	55	
Coupon 3	44.5	89			**Coupon 3**	28.9	58	
Mean of 3 Coupons	45.2	90	2.3		**Mean of 3 Coupons**	29.7	59	7.1
Spike Level: 5 µg/sample	Analyzed (µg/sample)	% Recovery	% RSD		**Spike Level: 5 µg/sample**	Analyzed (µg/sample)	% Recovery	% RSD
Coupon 1	4.0	80			**Coupon 1**	1.5	30	
Coupon 2	4.2	84			**Coupon 2**	1.0	20	
Coupon 3	4.1	82			**Coupon 3**	1.3	26	
Mean of 3 Coupons	4.1	82	2.3		**Mean of 3 Coupons**	1.3	25	16.2
		% Recovery	% RSD				% Recovery	% RSD
Average % Recovery (n=12)		88	5.0		**Average % Recovery (n=12)**		?	
In Example 3, the recoveries for the 125 µg/sample are above the < 110% recovery level and an investigation cannot improve the recovery (see below).					In Example 4, the recoveries and % RSD both fail, most likely because anodized aluminum has a porous surface.			

In Example 3, the recovery uses only the 9 samples, which are averaged. The high recoveries are intriguing. Based on the other recoveries, the high recoveries should be able to be improved. However, spending too much time on this investigation is probably not value added, since the high level is greater than the ARL and will fail cleaning while the accuracy is demonstrated to be near the ARL.

In Example 4, there are three options:

- Dedicate the piece of equipment and have one for each product manufactured on the equipment
- Replace the aluminum piece within the equipment with a piece made with a MOC with a smooth surface and repeat the recovery
- Accept the low recovery recognizing that the data will be less accurate and be prepared to justify why material does not leach out during subsequent manufacturing

Applying the RF in the ARL Calculation

Once the RF is established, it is applied to each sample. Using Example 1 (Table 12.1), the RF can be applied as part of the ARL determination or it can be applied to each sample after testing.

- If the RF is < 100%, use the RF as is.
- If the RF is > 100%, use an RF of 1.
- For TOC analysis include a correction factor for the % carbon of the analyte of interest.

Applied to the ARL

$$\text{ARL (µg/swab sample)} = \frac{\text{PDE (µg/day)} \times \text{MBS (mg)} \times \text{Swab Area (cm}^2\text{/swab sample)} \times \text{RF (0.89)}}{\text{MDD (mg/day)} \times \text{SA (cm}^2\text{)}}$$

Where: PDE = Permitted Daily Exposure product being cleaned
RF = Recovery Factor of product being cleaned
ARL = Acceptable Residue Limit (SL) product being cleaned
MDD = Maximum Daily Dose of next product
MBS = Minimum Batch Size of next product
SA = Surface Area of equipment train being cleaned

13 Appendix 2 – Example: Cleaning Residue Limits Calculations for a Shared Formulation Tank (Product A/B)

This appendix presents an example MACO calculation using a formulation mixing vessel in which two different liquid products are made one after the other in the same tank with a clean in between the two batch mixing operations (see Chapters 6 and 7).

The vessel is used to formulate the liquid product, mixing liquid-based API, excipient, and diluent. Only the API has pharmacological action.

The tank is a 1000 L cylindrical, dished (torispherical) bottomed SS tank with a propeller style mixer (see Figure 13.1). The tank has a 1:1 diameter:height ratio. See Table 13.1 for the tank parameters.

These tanks are usually fitted with various fittings such as pH probes, manways, and nozzles, which may have inherent dead legs or pose hard-to-clean areas. In this example, a rigorous surface area/volume calculation has not been performed to take into account these fittings, since the aim of this example is to illustrate the concepts behind the calculation of the MACO value in a shared tank.

A maximum working volume is taken as 90% of the total tank volume (typical specification) (thus equal to 900 L). The minimum volume of the tank contents to assure that the mixer blade is fully covered is taken as 100 L.

An example calculation of an individual swab cleaning limit has also been given. As discussed in Chapter 7, there are various swabbing strategies that could be implemented by users of the tank, such as assessing each swab value against the cleaning limit, taking average values of all swab readings, or assessing shared product areas separately from the non-product contact worst-case locations. The strategy to be used must be determined and documented in the VMP prior to the performance of the validation study.

In this example the MACO value for the cleaning process after Product A has been mixed and before Product B can be introduced has been calculated, and the total product contact area of the vessel has been considered.

Figure 13.1: Example Mixing Vessel

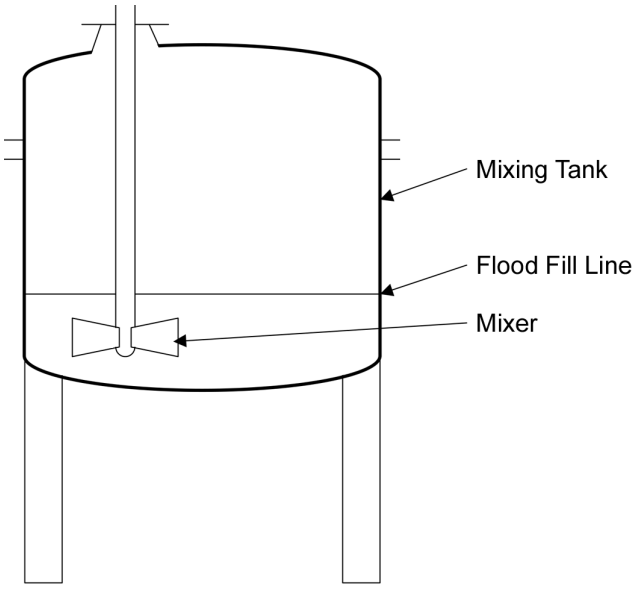


Table 13.1: Tank Parameters

Parameter	Measurement
Total Volume	1000 L
Working Volume (90% total volume)	900 L
Diameter	1 m
Total Height	1 m
Wall Thickness	2 mm
Volume of Torispherical Dished Base	99.2 L
Surface Area of Torispherical Dished Base	0.99 m^2
Agitator Surface Area (Blades)	0.2 m^2

In this worked example, the properties of the dished base have been calculated using the formulas specified in DIN 28105:2002 [115]:

Volume = $0.1\ (Do-2Tw)^2$

Surface area = $0.99\ Do^2$

Where: Do = Outside diameter of tank
Tw = Wall thickness

Note: These values are usually supplied by the tank manufacturer.

Product A:

Quantity: 900 kg

PDE value: 0.0005 mg/day (0.5 µg)

Product B:

Quantity: 700 kg

Standard Therapeutic Daily Dose: 100 mg/day

MACO is the mathematically calculated quantity of residue from Product A when carried over into Product B that **can** represent potential harm to the patient, and is based on the PDE value of Product A.

The following equation is used for the calculation of the MACO value:

$$\text{MACO (mg)} = \frac{\text{PDE or ADE} \times \text{MBS}}{\text{MDD or STDD}}$$

Where: MACO (Maximum Allowable Carryover) = Maximum acceptable transferred amount from Product A into Product B (mg)
PDE = Permitted Daily Exposure (mg/day)
ADE = Allowed Daily Exposure (mg/day)
MBS = Minimum Batch Size of Product B (mg)
MDD = Maximum Daily Dose (mg/day)
STDD = Standard Therapeutic Daily Dose (mg/day) for Product B

However the regulatory agencies expectation is that the cleaning residue SLs justified by HBELs are not intended to be used as the routine cleaning limits after the cleaning process is qualified [7, 27]. Cleaning control limits post-cleaning process qualification should be based on a user-defined fraction of the calculated MACO value (e.g., action limits and alert levels consistent with the cleaning process performance and capability).

In this example, the MACO value is calculated as:

$$MACO = \frac{0.0005 \text{ mg/day} \times 700 \text{ kg}}{100 \text{ mg/day}}$$

$$MACO = \frac{0.35 \text{ kg}}{100}$$

$$MACO = 3.5 \text{ g}$$

Instead of calculating each potential product change situation, the worst-case scenario can be chosen. Then a case with most active API (lowest PDE) is chosen to end up in the following API with the smallest ratio of batch size divided with STDD (MBS:STDD ratio).

Grouping

In some cases where there are a large number of dissimilar products (liquids, ointments, creams, gels, etc.) or products with a different route of administration (e. g. oral, topical, parenteral) being manufactured, they may be grouped together. This may allow a more representative determination of a worst-case product for each group, avoiding such situations as topical products being subjected to the stricter acceptance criteria applied to parenterals (which have significantly higher safety factors).

Note: All products within a group should be cleaned in the same way according to the same SOP.

Grouping is discussed in more detail in Section 5.6.

Matrix Approach

If the vessel is used to manufacture several different products sometimes with different batch sizes, it may be usefull to determine the acceptance criteria on a case-by-case basis (in the form of a matrix as shown in Table 13.2) to avoid unecessarilly strict acceptance criteria.

Table 13.2: MACO Value (g)

			Product A	Product B	Product C	Product D	Product E
		Batch Size	900	700	500	600	300
	PDE value (mcg/day)	STDD[1] (mg)	40	100	800	200	3000
Product A	0.5			3.5	0.3125	1.5	0.05
Product B	1.0		22.5		0.625	3.0	0.1
Product C	25.0		562.5	175		75	2.5
Product D	10.0		225	70	6.25		1.0
Product E	50.0		1125	350	31.25	150	

Note:
1. STDD = Standard Therapeutic Daily Dose

Swab Results

In terms of swab values, the total surface area of the vessel is used.

For instance, if the area swabbed was 25 cm^2 (= 0.0025 m^2), the MAC was 3.5 g (3500 mg), and the total surface area of the vessel was approximately 5.4 m^2 (vessel plus allowance for agitator surface, etc.), then the maximum residue per swab is:

$$\text{Per swab} = \frac{3500 \text{ mg} \times 0.0025 \text{ m}^2}{5.4 \text{ m}^2}$$

Per swab = 1.6 mg (assuming 100% swab recovery)

Interpretation of swab samples together with the determination of recovery values are described in detail in Section 7.1.

Final Cleaning Recommendation

In this example, a MACO value for Product A into Product B is 3.5 g. A maximum swab value (assuming 100% recovery) is 1.6 mg/25 cm^2.

Note: These calculated limits can be further optimized using risk-based strategies if they are scientifically justified and well documented. For example, the calculation of product contact surface area can be further refined for small batches in vessels that are well controlled, so as to avoid setting an extremely conservative low cleaning limit. These strategies are applied on a case by case basis, following regulatory guidance and company policies.

14 Appendix 3 – Example: Protocol for Development and Establishment of a Visible Residue Limit (VRL)

This appendix demonstrates the application of cleaning principles presented within this Guide. It is not meant to be a specification or a step-by-step process; instead, it is considered an example of how cleaning principles can be adapted and applied to a cleaning process.

This appendix illustrates an example of a cleaning validation protocol and describes approaches for the establishment of VRLs.

Table of Contents

1 Purpose

The purpose of this protocol is to develop and establish a Visible Residue Limit (VRL) for process residues and to qualify personnel for visual inspection of cleaned equipment. The study will include spotting decreasing amounts of residue on stainless steel coupons and having trained personnel determine whether they are able to visually detect the residue. It is recommended to have as many observers as practically possible to minimize variability among the observers. It is also recommended to use observers that are routinely involved in the visual inspection of cleaned equipment.

The final inspection of visually cleanliness for VRL purposes will be performed by a qualified person and verified by a second qualified person prior to equipment release for use.

2 Scope

This protocol will address process residues currently used at the site that disperse uniformly over surfaces. Determinations of VRLs and qualification of personnel will be on a priority basis as identified by the validation departments at each site.

3 Responsibilities

Validation

- The writing of this protocol, ensuring that it is technically accurate, and the acceptance criteria are valid.
- The execution of this protocol.
- Assembling the documentation and test results.
- Evaluating the protocol results and authoring a completion report.

Quality Control
- Providing coupons and residues.
- Providing solutions/suspensions of the residues.
- The execution of this protocol.

Quality Assurance
- Reviewing and approving protocols and the VRL data.
- Reviewing and approving completion report.

Operations
- Reviewing and approving this protocol.
- Reviewing and approving the completion report.
- Qualify personnel to perform VI.

4 Definitions

Coupons – Samples of manufacturing equipment product contact surfaces.

Visible Residue Limit (VRL) – The lowest concentration of dried residue that can be reliably seen by trained personnel under defined conditions.

5 Acceptance Criteria

The VRL will be determined for available analytes and finished products as available.

6 Materials

- Finished products as available
- API laboratory standards
- Detergent
- Stainless steel coupons
- Calibrated light meter
- Measuring tape or ruler
- Protractor
- Access to area overhead light control
- Analytical balance
- Laboratory solvents
- Laboratory fume hood
- Pipettes capable of pipetting 0.1 ml

7 Requirements

7.1 The VRL determination identifies the level at which personnel can reliably see residue as a measure of equipment cleanliness. VRLs will be compared to the cleaning ARLs to determine whether the VRLs are lower than the ARLs and can be implemented for cleaning monitoring. The VRLs will also be used to establish viewing parameters for qualification of personnel to perform visual inspections.

7.2 VRLs are determined by inspecting stainless steel coupons, at least 5 cm × 5 cm, spiked with the analyte. Each VRL determination will consist of at least 6 coupons spiked with decreasing amounts of the target analytes and a blank coupon spiked with solvent.

7.3 Analytes will be prepared as individual solutions or suspensions and spiked onto individual coupons.

7.4 VRL determinations will be established once for each target analyte or finished product. This VRL can be used at all manufacturing sites where the target analyte or finished product is manufactured.

7.5 Observers will view the coupons from a distance of 60 cm to set the VRL. (Equipment that is manually cleaned is usually visually inspected from approximately 60 cm.) Observers should be thoroughly trained to perform the coupon inspection.

7.6 Residues will also be viewed from distances up to 4.5 m, depending on distance viewing requirements at the manufacturing site. This will also determine the limits of the VRL viewing parameters for the qualification of personnel. (Larger fixed equipment are visually inspected from up to 4.5 m away as required by the equipment design and installation.)

7.7 Intermediate viewing distances can be used to determine limitations to the VRL viewing parameters.

8 Documentation

8.1 VRL determinations will be documented by individual observers using Appendix A – Visible Residue Limit Determination Form.

8.2 At the completion of the VRL determination activities, the completed Appendix A – Visible Residue Limit Determination Form, Appendix B – Visible Residue Limit Documentation Form, and the location key will be reviewed and approved by the Qualified Trainer or QA.

9 Procedure

9.1 The analyte (product, API, excipient or detergent) solution/suspension is prepared. The solvent used and the solution preparation will be documented in a laboratory notebook.

9.2 Record the balance, pipette and light meter ID numbers and calibration due date and the solvent batch number and expiration date in a laboratory notebook as applicable.

9.3 Solutions or suspensions of the target analyte (product, API, excipient or detergent) are prepared at 800 µg/ml, 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml concentrations as shown in Table 14.1 for a 100 µl spiking volume. Adjust the solution concentrations based on the optimal spiking volume as determined for the product/solvent combination. For example, a spiking volume of 200 µl is used for detergent; therefore, spiking solution concentrations would be half those in Table 14.1.

For dilutions, pipette 5 ml of Solution A into a 10 ml volumetric flask and bring to volume with solvent. The preparation of the solutions or suspensions will be documented in a laboratory notebook. Record the solvent used for the analyte preparation in the laboratory notebook.

Table 14.1: Example Spiking Preparation and Target Concentrations based on a 100 µl Spiking Volume

Spiking Solution Preparation	Spiking Solution Concentration	Residue Spiked
Solution A – 800 µg/ml	800 µg/ml	80 µg
Solution B – 5 ml of A into 10 ml	400 µg/ml	40 µg
Solution C – 5 ml of B into 10 ml	200 µg/ml	20 µg
Solution D – 5 ml of C into 10 ml	100 µg/ml	10 µg
Solution E – 5 ml of D into 10 ml	50 µg/ml	5 µg
Solution F – 2 ml of E into 10 ml	10 µg/ml	1 µg
Solvent	0 µg/ml	0 µg

9.4 Stainless Steel Coupons

Prior to use, all coupons will be inspected for appearance. Their appearance should be comparable to a new coupon. When necessary the coupons will be washed and allowed to air dry.

Coupons must be clean and scratch-free. If the coupons are still not clean or visibly scratched, replace the coupon.

Record the number and condition of the coupons in a laboratory notebook.

Clean coupons spiked with solvent will be used as blanks.

9.5 Spiked Coupon Preparation

9.5.1 Label the back of the coupons with the analyte name and concentration.

9.5.2 Each prepared solution or suspension will be used to spike clean coupons. Use a calibrated pipette to apply the appropriate volume (e.g., 100 µl) of the spiking solution onto a clean dry stainless steel coupon. Using the pipette tip as necessary, disperse the solution as a circle of approximately 5 cm in diameter.

Alternatively, use a coupon of a known dimensions with a lip that retains the solution. Add a quantity that covers the entire working surface and evaporate evenly to obtain an even film of a known concentration across a known area.

9.5.3 If the ARL from the Cleaning Limit Final Report is not encompassed in the prepared samples, prepare a separate coupon at the ARL of the product.

9.5.4 Allow the coupons to air dry for at least 1 h. Protect the coupons from dust during the drying process.

9.5.5 Once the coupons are dry, measure the area of each dried ring residue, calculate the concentration of the residue in µg/cm^2 and record in a laboratory notebook.

If the residue dries as a uniform film, divide the amount spiked by the known area of the coupon.

9.6 Spiked Coupon Presentation/Visual Qualification

9.6.1 For consistency, VRL determinations and Visual Qualifications are done at a distance of 60 cm. For the VRL determination the coupons are arranged in order of decreasing concentration on the flat surface of the laboratory bench.[23] If possible, the background for the coupons should be stainless steel but must be consistent for all coupons. The viewing angle is measured using a protractor, but a comfortable viewing angle at this distance is about 30°. The light level should be > 200 lux at the coupon surface.

9.6.1.1 A location key is documented by the qualified trainer using the Coupon ID table portion of Appendix A to establish the positions of the coupons for assessment of observer results.

9.6.1.2 VRLs of multiple analytes may be determined simultaneously and recorded in Appendix A.

9.6.2 A viewing station can be used to hold the coupons at 45° for the purposes of the VRL determination from greater distances. The maximum viewing distance should match the greatest viewing distance for the equipment in the manufacturing area. Other appropriate ways of positioning the coupons may be used. The viewing angle and light level need to be controlled and measured.

9.6.3 Multiple viewing distances, light levels, and viewing angles can be surveyed to determine the limits of the viewing parameters for the individual VRLs.

9.6.4 Take a photograph of the coupon presentation array for the VRL report. It should be noted that typically the picture will not be as sensitive as the observer determinations.

9.7 VRL Determination

9.7.1 VRLs are determined using a minimum of four observers to visually examine the spiked coupons. Include qualified personnel from the operations area if practical. Each operator inspecting equipment for cleanliness should be qualified against the visual threshold determination or the threshold should be amended as necessary.

9.7.2 Each observer records the results in Appendix A.

9.8 VRL Documentation

9.8.1 The lowest residue concentration seen by each observer is recorded in Appendix B.

[23] The presentation order of the coupons appears to have no effect on the determination of VRLs when the inspection is performed by trained observers. Both a sequential presentation from high to low concentration and a random presentation results in a rugged VLR determination [116].

9.8.2 The lowest residue concentration level seen by all observers is designated as the VRL. The lowest residue concentration level seen by all observers is used to determine the VRL. There needs to be a safety factor applied beyond what can be seen to fully account for the more challenging environment in production. This safety factor should be commensurate with the threshold to ensure a clear margin of safety.

9.8.3 If the lowest residue concentration tested is seen by all observers, prepare additional coupons at lower concentrations until the analyte is not visible.

9.8.4 At the completion of the VRL determination activities, the completed Visible Residue Limit Determination Form, Appendix A, and the Visible Residue Limit Documentation Form, Appendix B and will be reviewed and approved by the Executor or QA. The information to be reviewed and approved will also include the parameters (light intensity, observation angle, and observation distance) needed to transfer the VRL method to operations for regular monitoring purposes.

Appendix A – Visible Residue Limit Determination of Manufacturing Residues

Page _____ of _____*
**This page may be duplicated as needed.*

Name of Observer: _____ **Employee ID:** _____

Light Meter: Instrument No.: _____ **Calibration due date:** _____

Viewing Conditions			
Location		**Distance (m)**	
Coupons		**Angle (degrees)**	
Light Level (lux)			

Record Y for visible residue and N for not-visible residue.

Residue	1	2	3	4	5	6	7
A							
B							
C							
D							

Observer

Name	Signature	Date

Executor

Name	Signature	Date

Attach with Appendix B – Visible Residue Limit Documentation and the location key.

Appendix B – Visible Residue Limit Documentation of Manufacturing Residues

Page _____ of _____*
**This page may be duplicated as needed.*

Name of Executer:_____ **Employee ID:** _____

Viewing Conditions			
Location		**Distance (m)**	
Coupons		**Angle (degrees)**	
Light Level (lux)			

Record the lowest concentration seen by each observer for each residue from Appendix A.

Residue	Observer 1	Observer 2	Observer 3	Observer 4	VRL*
*VRL is the lowest concentration seen by all observers.					

Executor

Name	Signature	Date

Reviewed by

Name	Signature	Date

Attach with Appendix A Visible Residue Limit Determination and the location key.

Revision History

Revision Number	Date	Section	Description	No.
0		*N/A*	*Original Issue*	

15 Appendix 4 – Example: Bioburden Swab and Rinse Recovery Methods

This appendix describes a recommended step-by-step approach for developing a swab and rinse recovery method for bioburden. See Chapter 6 for further explanation.

15.1 Swab Recovery Method

15.1.1 Test Method Assessment

Sterilized coupons (small pieces of material representing equipment to be sampled, e.g., SS, glass, rubber, plastics and plexiglass) are needed. A coupon typically 5 cm × 5 cm (25 cm^2) is representative of a standard sample size for smaller irregular surfaces and larger flat surfaces. However, note that smaller sample surface areas are less sensitive for bioburden recovery.

To increase recovery the following factors should be considered:

- Evaluate the swab or contact method (direct method) for optimal recovery
- Ensure the swab material does not inhibit the extraction of test microorganism
- Evaluate the contact plate growth media (direct method) for optimal recovery for each test microorganism
- Evaluate inoculation solution or method for each challenged microbial
- Train laboratory technician on the sampling methods and extraction method
- Properly clean and sterilize the test coupons
- Verified that there was no chemical residue on the coupon that could interfere with the recovery study test results and method
- Use a recovery solution (inoculation) such as PBST or other effective solution to spread, stabilize, and prevent desiccation (drying) of inoculated (spiked) microorganisms on coupons
- Growth promotion results of sampling media were acceptable
- Apply proper serial dilution method for each test microorganism
- Consider negative and positive controls
- Verify that test microorganisms were not contaminated
- Clean and sterilize all test equipment, materials, and sampling tools
- Write laboratory recovery study protocols
- Select proper incubation temperature and time
- Verify that laboratory conditions will not impact the recovery, i.e., during study preparation, the use of surface disinfection aerosol did not accidentally contaminate the test coupons

- Perform the study in a clean environment such as within a Biosafety Cabinet
- Spiked (inoculation) microorganism on test coupons are not allowed to dry too long (validated drying time) or consider using a wet method

15.1.2 Preparation of the Working Cultures

Before starting the bioburden recovery studies, it is important to verify the growth promotion properties of the intended media using the challenge microorganisms listed in Table 15.1 [117]. The challenge microorganisms for the test method study should include a Gram negative rod, Gram positive cocci, yeast, mold, a spore former, as well as the most common and virulent (resistant) environmental isolate. However, from a cleaning risk and process control perspective, the study is considered more representative if environmental isolates are used. If a company has not developed an extensive catalog of their environmental isolates, then the representative microorganisms in Table 15.1 should be considered until an environmental database is developed.

Table 15.1: Representative Challenge Organisms

Challenge Microorganism	ATCC® [118]	Organism Type
Staphylococcus aureus	ATCC® 6538™	Gram positive coccus
Pseudomonas aeruginosa	ATCC® 9027™	Non-fermenting, Gram negative rod
Bacillus subtilis	ATCC® 6633™	Gram positive spore-forming rod
Candida albicans	ATCC® 10231™	Yeast
Escherichia coli	ATCC® 8739™	Fermenting, Gram negative rod
Environmental Isolate Sp.	EM isolate	Gram negative rod, Gram positive coccus or yeast, fungi, etc.
Aspergillus brasiliensis	ATCC® 16404™	Filamentous fungus
ATCC® = American Type Culture Collection		

Reconstitute the ATCC® [118] cultures according to the vendor's instructions or internal laboratory procedures. After the challenge microorganisms have been prepared,

> *"…transfer them onto Tryptone soya medium (agar or broth) and incubate at 30-35°C for a minimum of 48 h. Inoculate yeast cultures onto Sabouraud's dextrose agar (SDA) medium (agar or broth) and incubate at 20-25°C for 2-3 days. Inoculate filamentous fungi (mold) culture onto SDA and incubate at 20-25°C for 5-7 days or until good sporulation is achieved."* [108]

Table 15.2 contains the microorganisms incubation temperatures and periods.

Table 15.2: Challenge Microorganisms Growth Requirements

Challenge Microorganism	ATCC® [118]	Atmosphere	Incubation Time	Incubation Temperatures
Staphylococcus aureus	ATCC® 6538™	Aerobic Growth	2–3 days	30°C–35°C
Pseudomonas aeruginosa	ATCC® 9027™	Aerobic Growth	2–3 days	30°C–35°C
Bacillus subtilis	ATCC® 6633™	Aerobic Growth	2–3 days	30°C–35°C
Candida albicans	ATCC® 10231™	Aerobic Growth	2–3 days	30°C–35°C
Escherichia coli	ATCC® 8739™	Aerobic Growth	2–3 days	30°C–35°C
Environmental Isolate Sp.	EM isolate	Aerobic Growth	TBD	TBD
Aspergillus brasiliensis	ATCC® 16404™	Aerobic Growth	5–7 days	20°C–25°C
ATCC® = American Type Culture Collection, TBD = To Be Determined				

> *"After the specified incubation period, harvest the bacteria and yeast cultures by washing each plate with approximately 2 mL of sterile USP Saline Test Solution (TS), pH 7.0 buffered sodium chloride solution or pH 7.2 phosphate buffer. Carefully, centrifuge the culture liquid media until a microbial pellet forms at the bottom of the test tube, remove the supernatant and re-suspend the microbial pellet in sterile USP Saline Solution.*
>
> *Using a spectrophotometer, adjust the bacterial suspensions using buffer diluent to an optical density of 0.1-0.3 at a wavelength of 550 nm; adjust yeast suspensions using buffer diluent to a 5.0 McFarland turbidity standard."* [119, 117]

Using a standard serial dilution method, prepare a 1 ml aliquot of the 10^5 or 10^6 dilution for a standardized suspension of bacteria and a 1 ml aliquot of the 10^4 dilution for a standardized suspension of yeasts. This dilution factor should yield counts in the range of 10–100 CFU [108].

> *"If the standardized inocula of bacteria and yeasts are not used promptly (within 2 hours), the suspensions should be stored under refrigeration. Suspensions of vegetative organisms prepared in USP Saline TS or a buffer solution remain viable and stable for 7-10 days if maintained under refrigerated conditions."* [108]

15.1.3 Spore Suspension Preparation

Harvest mold spores (*Aspergillus brasiliensis*) by washing the agar surface with sterile USP Saline TS or a buffer solution containing 0.05% polysorbate 80. Use a sterile inoculating loop or sterile glass beads to release the spores and combine the washings in a sterile container. Harvest the bacterial spore suspension (e.g., *Bacillus subtilis*), by preparing the inoculate agar plates with sterile water and heat shock the suspension for 15 min at 65°C–70°C. Start timing when the temperature reaches 65°C [108, 119]. Rapidly cool the suspension in an ice bath (0°C–4°C) and store under refrigeration until needed.

15.1.4 Swab Sampling Procedure

The test method steps below are based on a study performed by Eissa and Mahmoud [108] with a few modifications to test method.

Explanation of techniques:

> ***"Agar Streaking Technique:** After sampling swab was streaked on solid agar media."* [108]

Prolonged Vortexing: Vortexing for 30 s followed by 2 min

> ***"Outward Flushing Technique:*** *Swab after vortexing was subjected to washing by flushing diluent from inside of the swab hollow stick to outside."* [108] (The % recovery of bioburden from swab outward flushing – applying pressurized flushing by sterile pumping device with buffer pH 7 at the internal side of the swab – criteria for all tested surfaces and all microorganisms.)
>
> ***"Recovery Study Using the Wet Method Followed by Dry Swab Method:*** *This procedure was performed for vegetative cells (bacteria and fungi) to prevent loss of viability due to desiccation."* [108]

Pipette 1.0 ml of the TS into a sample container, and *q.s.* with recovery solution to make a 40 ml sample. Label this sample as Wet Positive Control, and ensure recovery solution and test microorganisms in control sample are identified for each sample. Repeat for each TS and each recovery solution. Calculations to determine the RF for the sampling procedure are based on the results from this sample.

- pH 7.0 buffered sodium chloride solution or pH 7.2 phosphate buffer
- Sterile USP Saline TS or a buffer solution containing 0.05% polysorbate 80 (higher recovery rate for most microorganisms)

Label each sample vial according to the swab recovery solution, challenge microorganism, and wet or dry swab methods.

Sample Each Coupon per the Following Steps

1. Always wear sterile powder free gloves when handling swabs, vials, and coupons.
2. Preform study under laminar flow unit using sterile tools and components.
3. Obtain a sufficient quantity of commercially available sterile vials containing diluent fluid and one unopened bag of sterile swabs. Obtain a sterile pair of snips (scissors). Place the snips in the beaker of sterile diluent solution.
4. Carefully remove a swab from the plastic bag and ensure the swab tip does not contact any surface or foreign material before swabbing coupon to avoid contaminating sample. Hold the swab by the handle end.
5. Place the swab vial on a flat surface and carefully remove the cap. Carefully handle the sample vial and cap so that nothing, including gloved hands, comes in contact with the interior of the container or cap.
6. Completely immerse the tip of the swab in the vial containing the sterile diluent solution. The diluent solution is: buffer solution containing 0.05% polysorbate 80 (diluent solution).
7. Press the tip of the swab against one of the inner walls of the vial to eliminate excess solution from the swab.
8. Carefully lift the swab tip out of the vial. The swab tip should be thoroughly saturated, but not dripping with liquid.
9. Apply the swab to the surface to be sampled in the following manner:
    a. Using a back-and-forth motion, rub one of the flat sides of the moistened swab over the sampling area. Continue rubbing the swab on the sampling area until the entire area has been covered.
    b. Turn the swab over, and using the opposite flat side, rub the sampling area in a back-and-forth motion that is perpendicular to the previous sampling sweep. Continue rubbing the swab on the sampling area until the entire sampling area has once again been covered.

If a square swab area is not available due to equipment configuration restrictions, swab the same size area over a rectangular or equivalent pattern as shown in Figures 15.1 and 15.2.

Figure 15.1: Swabbing Irregular Surfaces

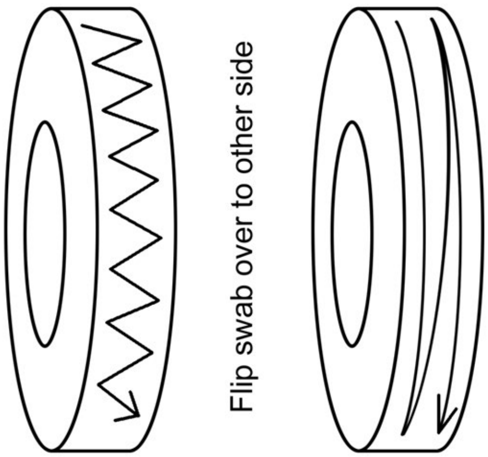
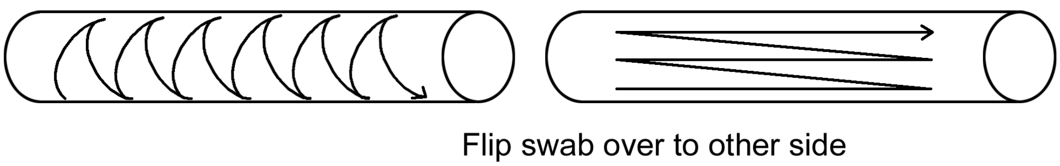


Figure 15.2: Swabbing Inner Surfaces – Process Pipe



15.1.4.1 *Recovery Study Using the Dry Swab Method*

Inoculate each type of coupon surface with 25–100 CFU[24] of the inoculum's suspension prepared in sterile saline solution using a micropipette. Using a disposable sterile plastic bent rod (hockey stick shape), evenly spread the suspension onto the coupon for a contact time of less than 1 min (see Figure 15.3). Following the method described in Section 15.1.4 for swabbing surfaces, use a dry swab method to recover each test organisms from the liquid inoculum's surface of the coupon.

1. Initial and date the label on each vial after ensuring the sample is fully identified. Submit all samples for bioburden testing.

2. Place swab into each vial containing 50 ml of swab diluent solution. Replace the cap on the sample vial and tightly seal. Process per method in Item 3.

3. Vortex the swab + diluent solution for 30 sec, followed by an additional 2 min. Using the outward flushing technique method, aseptically remove swab and process swab diluent solution sample using membrane filtration method and inoculate onto TSA with lecithin and polysorbate 80 for bacteria and SDA for fungi with an incubation condition at 30°C–35°C for 2 to 3 days for aerobic bacteria, and 20°C–25°C for 5 to 7 days for fungi.

4. Results should be reported as the number of CFU per swab and the % recovery for each test organism calculated. Perform the swab sampling method in triplicate for each challenge organism.

5. Prepare Swab Negative Controls. For the Dry Swab Method Blank, place a swab into a swab vial then *q.s.* to 50 ml with diluent solution and label as Dry Swab Blank. Prepare negative 2 controls for each swab method (Dry and Wet).

[24] Typically, ≈ 100 CFU microorganisms is used; the higher the CFUs the better the recovery rates.

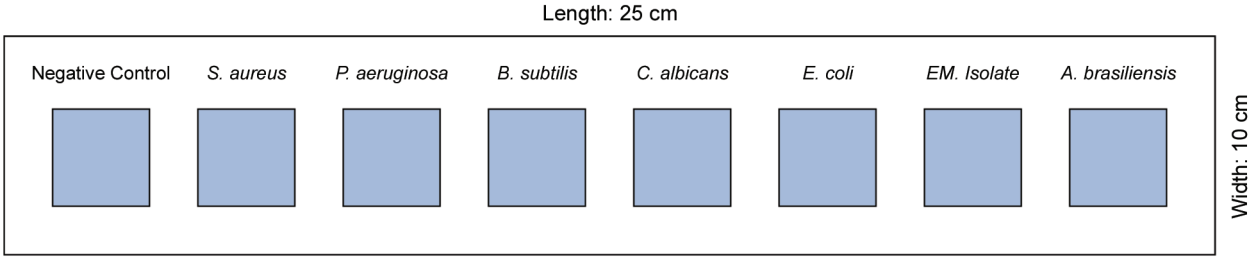
6. Prepare Swab Positive Controls. For the Dry Swab Method Blank, place an unused sterile swab into a sterile vial, transfer 1 ml (containing 100 CFU of the 7 challenge microorganisms) into 7 vials containing the diluent solution, then *q.s.* to 50 ml with diluent solution, and label each Swab Positive Control with the microorganism type. Process the sample according to Item 3 above.

7. For the diluent solution, use a Coupon Blank. Swab the untreated coupon with a wetted swab for the diluent solution used in this test. Do this for each coupon material used (Negative Control for Surface Material). Follow swabbing procedure described in Item 3 above.

8. A test-negative control for each swab is applied to verify aseptic manipulations by carrying out the procedure as described with uninoculated coupons. The inoculums solution level counts for positive control for microbial type should be ≥ 100 CFU and microbial growth should indicate no growth from the negative control samples.

Figure 15.3 is the inoculate method for all material types using a 5 cm × 5 cm (25 cm^2 sample area on sterile length 25 cm × width 10 cm) example:

Figure 15.3: Inoculate Method for All Material Types



Note: The above is one method that can be used to reduce the number of coupons for each challenge microorganism. However, its acceptable to have eight coupons of the same material for each challenge microorganism.

15.1.4.2 Recovery Study Using the Wet Swab Method

Repeat the study in Section 15.1.4.1 for a different material type of coupon surface.

In recovery study using the wet and dry method by swab technique, the inoculum's volume on the surface should be not less than 100 ul for sterile swab used for this study. Acceptance criteria for the bioburden % recovery from coupon surface should be not less than 50% of the inoculum's control.

It is important to remember that dry surface area is more consistent with a typically cleaning process; therefore, drying the coupon before sampling is important. Also, studies have shown higher recovery rates when a two-swab method is used – wet followed by dry swab. Both swabs are placed into the sampling dilution solution process using the method described above.

If recovery rate is low for a specific type of microorganisms (spore former or *Staphylococcus*) consider adding sterile 0.5% Tween® 80 to the saline solution. This should increase the % recovery.

15.2 Contact Plates Recovery Method

15.2.1 Overview of Different Contact Plate Types

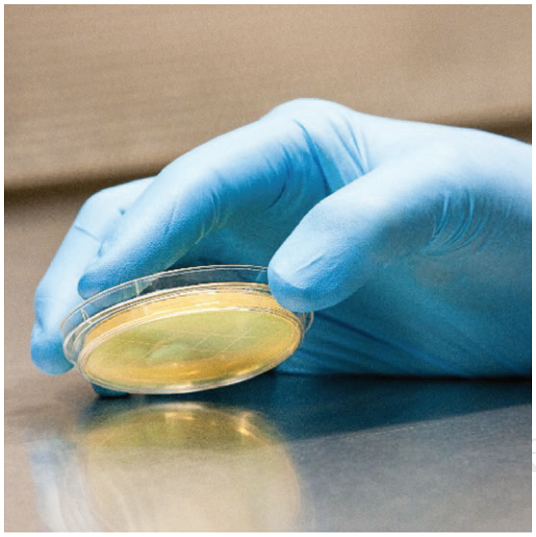
There are two types apparatus of contact plates and slides. The contact plate method is recommended when quantitative data are sought from flat, impervious surfaces. Contact plates are filled so that the media forms a dome. The nutrient medium used in the contact plate may also contain a neutralizing agent. The surface of the media is pressed against the surface being tested. The resulting sampled area for a 50 mm plate is approximately 25 cm^2. The plates are incubated for the required amount of time, and colonies, if present, are then counted.

Contact plates (Figure 15.4) can be used for microbiological monitoring of all types of surfaces with the exception of small irregular surfaces. Large flat surfaces, for example, are SS vessels or other end products, and the control of bioreactors, carts, containers, etc.

The advantage of contact plates is that they are very suitable for use in the industrial cleaning area. With the help of these plates, the analyst has a simple and quick method to check all kinds of surfaces for microbiological contamination and the hygienic status of the surface.

The disadvantages of the contact plate method are that this method is not suitable for sampling of irregular surfaces, confluent growth of microorganisms can occur, and the media residue must be removed from the sampling site. Colony overgrowth makes enumeration difficult on heavily contaminated surfaces.

Figure 15.4: Contact Plates
Used with permission from Bio-Med QC, LLC, https://www.biomedqc.com/.



Contact Slides are easy to use and provide flexible microbiological monitoring of flat and curved for both dirty and clean surfaces. Their unique design makes them a universal tool for curved surfaces such as large process piping, etc. Contact Slides media can be total count media as well as selective media for yeasts and molds, and for coliform bacteria.

15.2.2 Recovery Study Using the Contact Method

For contact plates, perform the study using the wet and dry method. The wet method allows the inoculation solution to contact the coupon surface for less than 1 minute. For the dry method, follow the recovery method for the wet and dry method listed above. See Sections 15.1.4.1 and 15.1.4.2.

15.2.2.1 Wet Method Contact Plates

On sterile 25 cm × 10 cm coupon materials, draw a 25 cm^2 circle the same diameter of the contact plate media surface area. Inoculate each type of coupon surface with 25–100 CFU of the inoculum's suspension prepared in sterile saline solution using a micropipette. Using a disposable sterile plastic bent rod (hockey stick shape), evenly spread the suspension onto the coupon for a contact time of less than 1 min.

Remove the lid and invert the primary plate. Line up the orientation of the plate media with the circle on the coupon. Lower the plate so that the surface of the agar is in contact with the coupon surface (circle with spiked challenged microbial).

Lightly but evenly press down with your finger tips on the back of the primary plate and then carefully lift the primary plate away from the coupon. Replace the lid on the plate. After sampling the coupon, incubate the plate at 30°C–35°C for 2 to 3 days for aerobic microorganisms, and 20°C–25°C for 5 to 7 days for fungi. Read contact plates results as CFU/25 cm^2.

Repeat this study in triplicate. Figure 15.5, the inoculate method for all material types, shows a 5 cm × 5 cm (25 cm^2 sample area on sterile length 25 cm × width 10 cm).

15.2.2.2 Dry Method Contact Plates

Using the sample coupon setup as above perform Dry Method Contact Plate recovery study. Inoculate each type of coupon surface with 25–100 CFU of the inoculum's suspension prepared in sterile saline solution using a micropipette. Using a disposable sterile plastic bent rod (hockey stick shape), evenly spread the suspension onto the coupon and allow the inoculum to evaporate to dryness under laminar flow conditions.

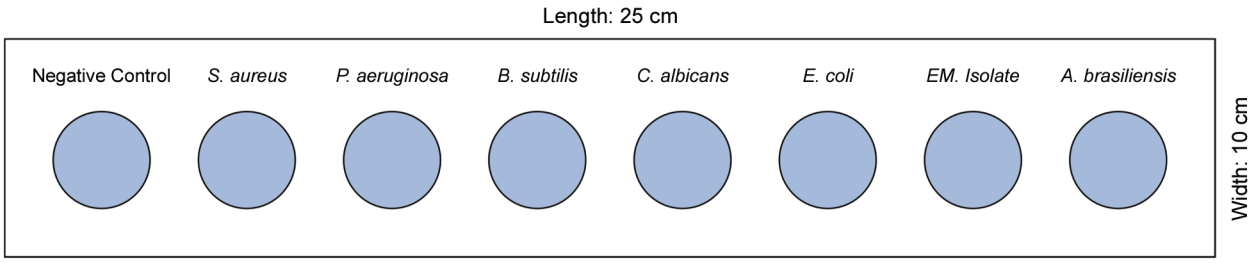
Remove the lid and invert the primary plate. Line up the orientation of the plate media with the circle on the coupon. Lower the plate so that the surface of the agar is in contact with the coupon surface (circle with spiked challenged microbial).

Lightly but evenly press down with your finger tips on the back of the primary plate and then carefully lift the primary plate away from the coupon. Replace the lid on the plate.

After sampling the coupon, incubate at 30°C–35°C for 2 to 3 days for aerobic microorganisms, and 20°C–25°C for 5 to 7 days for fungi. Read the contact plates results as CFU/25 cm^2.

Figure 15.5, the inoculate method for all material types, shows a 5 cm × 5 cm (25 cm^2 sample area on sterile length 25 cm × width 10 cm).

Figure 15.5: Contact Plate Diagram Spiked with Challenge Organisms



15.3 Rinse Water

15.3.1 Recovery Study Using the Rinse Method

The rinse method preparation procedure is similar to the swab methods but instead of using one large coupon, inoculate eight sterile coupons (25 cm^2), one for each challenge microorganism.

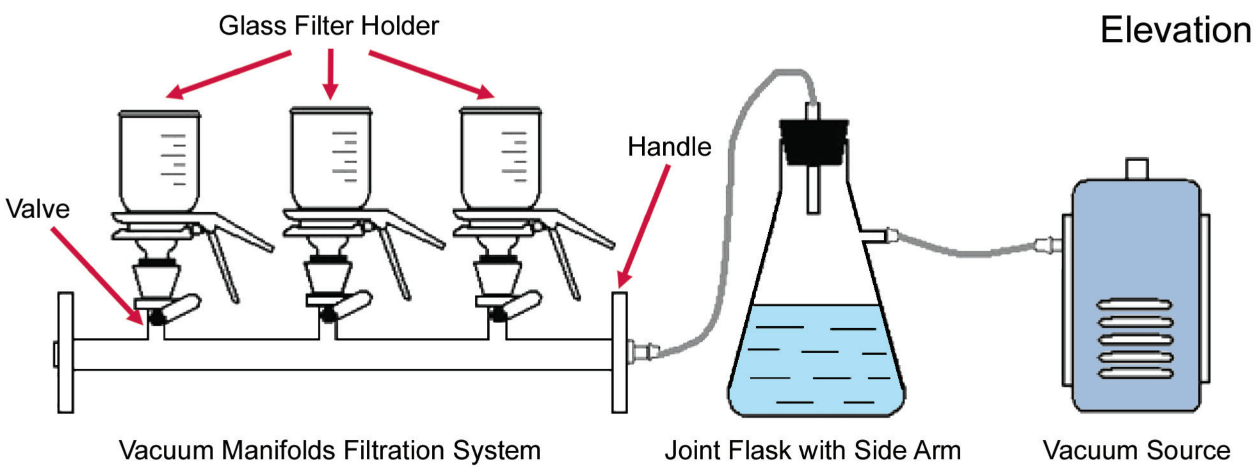
Inoculate the coupon per the procedure in Section 15.1.4.1 or 15.4.1.2 (wet/dry method).

Note: Some microorganisms may lysis when inoculated in water; therefore, select the appropriate test microorganisms for this study.

Using sterile forceps, aseptically transfer the coupon in an aliquot of sterile 100 ml or 200 ml diluents (e.g., purified or WFI water) contained in a sterile 250 ml flask, which would typically be used to rinse the given piece of equipment. Prepare eight sterile 250 ml flasks, for one each challenge microorganism plus the negative control (unspiked coupon).

Place the flask onto shaker for 5 min and then aseptically remove the coupon. Aseptically pour the diluent through a 0.45 µm membrane filter. Aseptically remove the filter and place onto a TSA agar or R2A media plate. See Figure 15.6.

Figure 15.6: Membrane Filtration Method Setup



Incubate the plates at 30°C–35°C for 2 to 3 days microbial and 20°C–25°C for 5 to 7 days for fungi. The recovered colonies are enumerated, and results are reported CFU/ml. Perform this procedure (rinse sampling) in triplicate for each challenge organism and material type.

A test-negative control for each study is used to verify aseptic manipulations by carrying out the procedure as described above using an uninoculated coupons. Test should be repeated if the inoculums level counts exceeded 100 CFU and microbial growth was recovered from the negative control samples. Recovery may vary because of the hydrophobic and hydrophilic cell walls of some of the test microbials.

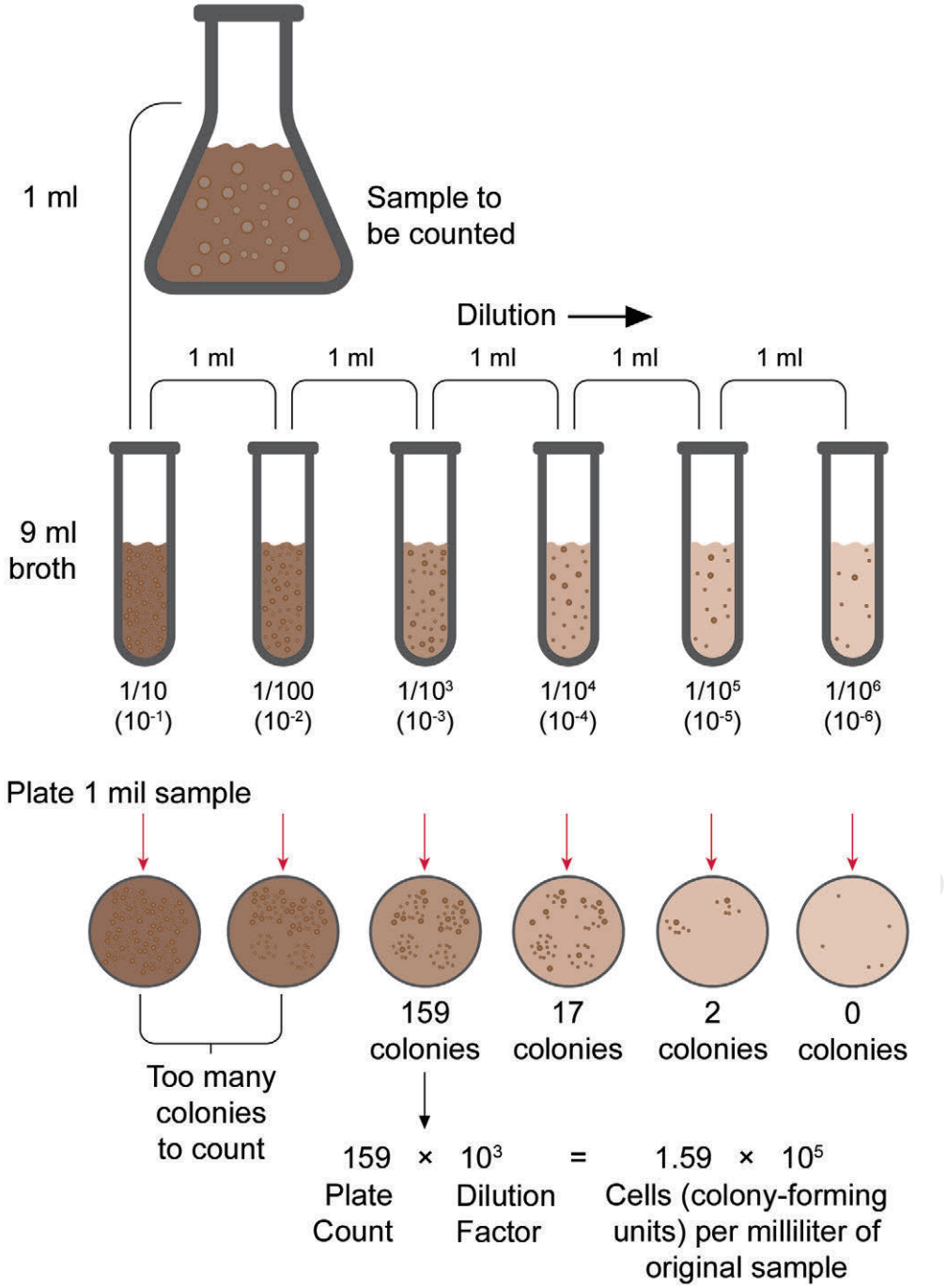
15.3.2 Diluting Samples, Plating, and Incubating

The cleaning process should show effectiveness in reducing bioburden and endotoxins to an acceptable level. Consequently, for sterile injectable products, it is important to measure bioburden and endotoxin levels before and after cleaning. In some cases, when samples are taken the microorganisms on the agar plate are too numerous, often when sampled during the pre-cleaning or dirty hold phase. The CFUs that are individual colonies will blend together, making them impossible to count. To solve this problem, samples are mixed into a liquid medium (sterile PW, ringer solution, or diluting fluid), then a small amount of that mixture is further diluted (i.e., serial dilution method).

This process is repeated six or more times to get an accurate reading of the CFUs. The final dilution is spread on an agar plate, incubated for 3 to 7 days at two temperatures (20°C–25°C for molds and yeast, and 32°C–35°C for bacteria), and then the colonies are counted (see Figure 15.7).

The objective of the serial dilution method is to estimate the concentration (number of colonies, organisms, bacteria, or viruses) of an unknown sample by counting the number of colonies cultured from serial dilutions of the sample, and then back track the measured counts to the unknown concentration.

Figure 15.7: Serial Dilutions Technique for Challenge Organisms



16 Appendix 5 – Example: Endotoxin Swab and Rinse Recovery Methods

This appendix describes the recommended step-by-step approach for developing a swab and rinse recovery method for endotoxin. See the explanation in Chapters 5 and 8.

16.1 Endotoxin Swab Recovery Method

Test Method Reagent

Reagents:

- USP Endotoxin Reference Standard (RS) – if required. Follow the manufacturers' directions for reconstitution and storage of standard endotoxins.
- LAL Reagent Water (LRW) (water for Bacterial Endotoxins test) – if dilutions of specimens or standard endotoxin are required

Endotoxin Stock Solution Preparation

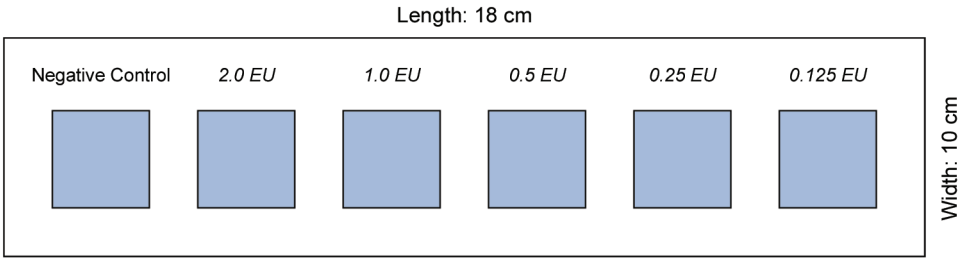
A Standard Endotoxin Stock Solution is prepared from a USP Endotoxin RS. After vigorously mixing the Standard Endotoxin Solution, prepare appropriate serial dilutions, (2 EU, 1 EU, 0.5 EU, 0.25 EU and 0.125 EU) using Water for BET. Use dilutions as soon as possible to avoid loss of activity by adsorption.

Coupon Preparation

Coupon extractions are performed using water free of detectable endotoxins. The extraction process involves spiking coupon material with different concentrations of endotoxins. The swabbing technique is similar to one used for the microorganism challenge.

Spike endotoxin concentrations onto the coupon as indicated in Figure 16.1, which shows a 5 cm × 5 cm (25 cm^2 sample area on sterile length 25 cm × width 10 cm).

Figure 16.1: Stainless Steel Coupon Surface Spiked with Endotoxin Units



Sample each coupon per the following steps:

Always wear sterile powder free gloves when handling swabs, vials and coupons.

Obtain a sufficient quantity of commercially available sterile vials containing diluent fluid and one unopened bag of sterile swabs. Obtain a sterile pair of snips (scissors). Place the snips in the beaker of sterile diluent solution.

1. Carefully remove a swab from the plastic bag and ensure the swab tip does not contact any surface or foreign material before swabbing coupon to avoid contaminating sample. Hold the swab by the handle end.

2. Place the swab vial on a flat surface and carefully remove the cap. Carefully handle the sample vial and cap so that nothing, including gloved hands, comes in contact with the interior of the container or cap.

3. Completely immerse the tip of the swab in the vial containing the sterile LRW (Sterile Endotoxin Free Water).

4. Using a back-and-forth motion, rub one of the flat sides of the moistened swab over the sampling area. Continue rubbing the swab on the sampling area until the entire area has been covered.

5. Turn the swab over, and using the opposite flat side, rub the sampling area in a back-and-forth motion that is perpendicular to the previous sampling sweep. Continue rubbing the swab on the sampling area until the entire sampling area has once again been covered. Repeat swabbing method using a dry swab.

6. Insert both swab tips into the 40 ml diluent solution vial. Cut the shaft of each swab right above the swab tip.

7. After swabbing each coupon, fill each vial with 40 ml of swab diluent solution. Replace the cap on the sample vial and seal tightly.

8. Prepare Swab Negative Controls. For the Wet Swab Method Blank, place a snipped swab into a swab vial then *q.s.* to 40 ml with diluent solution and label Wet Swab Blank.

9. For diluent solution, a Coupon Blank is required. Swab the untreated coupon with a wetted swab for the diluent solution used in this test. Do this for each coupon material used, (negative Control for Surface Material) Follow swabbing procedure above.

10. Prepare Swab Positive Controls. For Swab Method Blank, place a snipped swab into a swab vial then *q.s.* to 40 ml with diluent solution and label Positive Control for each EU Concentration.

11. Initial and date the label on each vial after ensuring the sample is fully identified. Submit all samples for endotoxin testing.

12. Vortex swab and diluent solution for 30 sec, followed by an additional 2 min, and test according to internal process for LAL gel-clot or LAL kinetic turbidimetric method.

16.2 Endotoxin Rinse Recovery Method[25]

During cleaning validation, it is important to sample and test both the initial and final rinses for endotoxin content. Ideally, a reduction in the level of endotoxin from initial rinse sample to final rinse water sample should be seen. It is important to note that rinse water sampling to only compendial methods without assaying for a specific residue is not acceptable to the regulatory agencies [17, 13]. Rinse water sampling, in combination with other sampling techniques, can provide a more complete assessment of cleaning efficacy than surface sampling. Typically, rinse water comes in direct contact with all product surfaces; therefore, it is considered an acceptable sampling method.

The rinse method preparation procedure is similar to the swab methods but instead of using one large coupon, inoculate six sterile coupons (25 cm^2), one for each of the endotoxin concentrations and one for the negative control.

[25] The rinse recovery studies need to replicate the production rinse techniques. Variables used in this example are for illustrative purposes only. Each study should adopt the appropriate parameters in accordance with their production methods.

Inoculate the coupon per procedure above (wet/dry method) and using sterile forceps, aseptically transfer the coupon in an aliquot of sterile 100 ml diluents (e.g., purified or WFI water) contained in a sterile 250 ml flask, which would be typically used to rinse the given piece of equipment. Prepare six sterile 250 ml flasks, for one each concentration of endotoxin including the negative control (unspiked coupon).

Place the flask onto shaker for 5 min and using sterile forceps, aseptically remove the coupon. Initial and date the label on each vial after ensuring the sample is fully identified. Submit all samples for endotoxin testing. Report results as number of EU per ml.

17 Appendix 6 – Case Study: Establishing Process Parameters for a Manual Cleaning Process

This appendix reinforces the information presented in Chapter 3 Risk Management in Cleaning, Chapter 5 Cleaning Methodologies, and Chapter 9 Equipment Issues and Challenges.

This case study is not meant to be a specification or a step-by-step process; instead, it should be considered an example of how cleaning principles can be adapted and applied to a cleaning process.

17.1 Introduction

The purpose of this case study is to define appropriate parameters for the manual cleaning of various equipment parts, utensils, and tools routinely used throughout pharmaceutical manufacturing operations. The equipment is dedicated to the manufacturing of two different products, used in campaign mode. A product changeover procedure is used between campaigns.

The ancillary equipment, equipment parts, utensils, and tools described herein are not single-use, disposable, nor are they dedicated to the manufacturing of a single product. They are cleaned for reuse in future batches of Product A or Product B.

The wash room in the production area is designed with one-way flow: ‘‘dirty in’’ moving to ‘‘clean out,’’ and is equipped with:

- Potable water line
- Pressurized hot potable water spray line
- Medium sized adjustable-setting sonicator (sonic bath) with a timer
- Purified Water, USP [46] spray line
- Clean dry compressed air line
- Heated drying rack area
- Timer

In addition, there is an area where clean dry parts can be wrapped or bagged and labeled prior to being moved to the clean equipment storage area.

For this case study, the manufacturing area does not have a parts washer and must perform manual cleaning.

17.2 Description of Parts and Tools to Be Cleaned

- Scoops are used in dispensing for measuring dry powder starting materials. These are stainless steel and come in three sizes: small, medium, and large.
- Spatulas are used for stirring, or removing dry or wetted powders or granulate blends. The MOC are Teflon™ or stainless steel, and come in a variety of sizes.
- Stainless steel 25 L vessel with lid used in the preparation of the coating solution
- Portable propeller-type mixer on stand. MOC of impeller blades and shaft is stainless steel with motor housing and electric plug affixed to a stand with locking wheels.
- Rotary tablet compression machine parts, including:
  - Punches and dies (see Figure 17.1)
  - Powder blend hopper and lid
  - Powder feeder system composed of the feeder system housing (gear box) and feeder paddles (cog wheels) (see Figure 17.2)

MOC for punches and dies is stainless steel with silicon bellows. The feeder system housing and feeder paddles are stainless steel, aluminum, and brass, and the powder hopper and lid are stainless steel.

In summary, the inventory of parts and tools to clean is diverse with different MOC (stainless steel, Teflon™, brass, and silicon), sizes, and uses.

Figure 17.1: Tablet Compression Machine Parts – Dirty and Clean Punches *(shown here material of construction = stainless steel)*
Courtesy of Fette Compacting GmbH, Grabauer Str. 24, 21493 Schwarzenbek, www.fette-compacting.com. Image rights remain the property of Fette Compacting GmbH.

Figure 17.2: Feeder System for a Tablet Compression Machine with Gear Housing and Feeder Paddle *(shown here material of construction = aluminum and brass)*
Courtesy of Fette Compacting GmbH, Grabauer Str. 24, 21493 Schwarzenbek, www.fette-compacting.com. Image rights remain the property of Fette Compacting GmbH.

17.3 Equipment Design Points to Consider

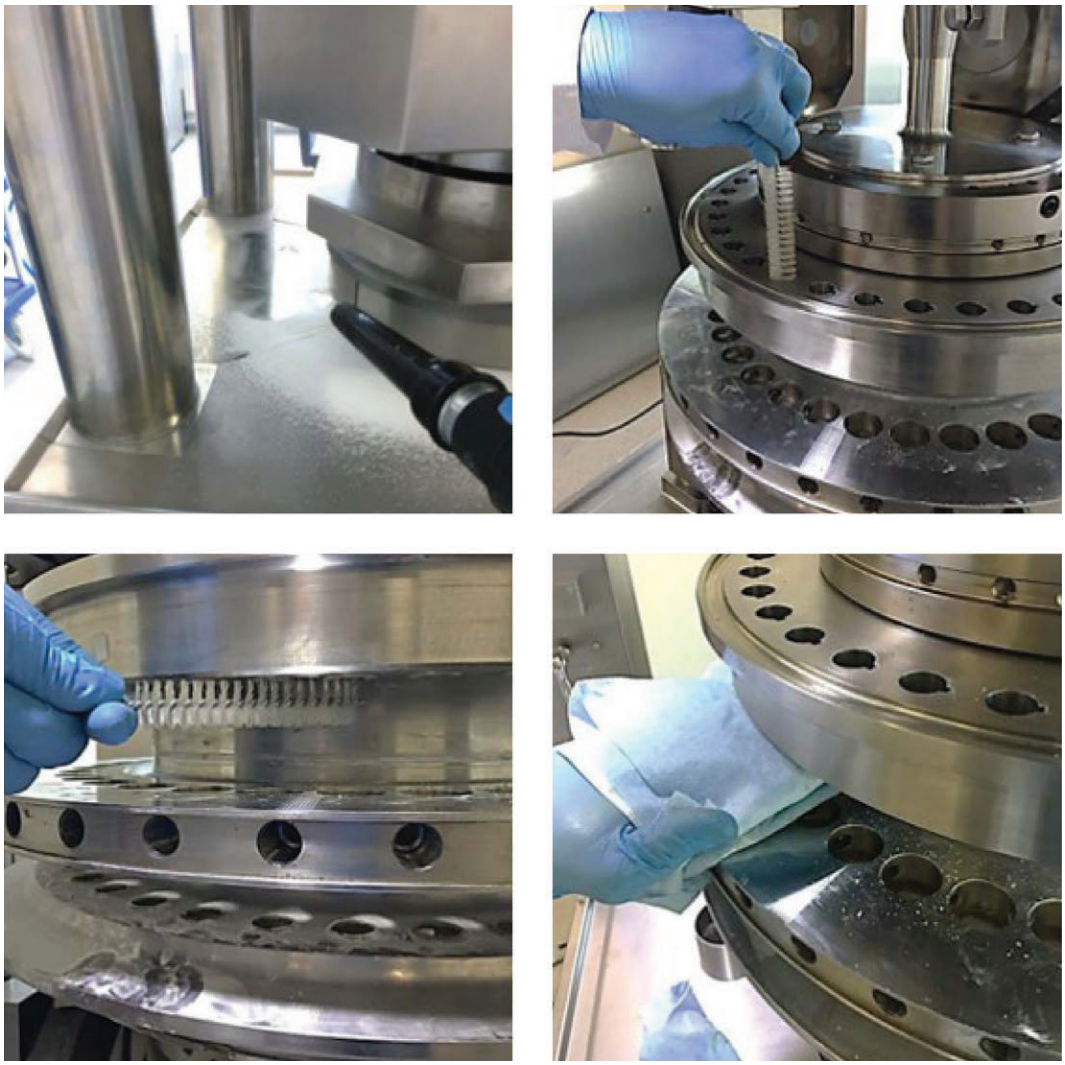
The scoop, spatula, and stainless steel pot and lid are not complex designs and allow for easy cleaning based on their basic shape and contours. All product surface areas are accessible by hand. Their design also makes visual inspection easy as there are no hidden surfaces or blind spots, and no disassembly is required. Attention needs to be paid to the edges and undersides of the pot and lid.

The motor housing of the portable propeller-mixer and the plug with cable are not product contact parts; however, they should be wiped clean with a damp cloth. The impeller blades and shaft need to be thoroughly cleaned, but being on a wheeled stand may dictate where this manual cleaning operation can be performed. For example, it is not possible to put this equipment into a small sink. It must be cleaned in an upright position by spraying with water in an area with proper drainage.

There are different change parts for the tablet compression machine with varying MOC, such as the feeder paddles or silicone upper punch bellows, so compatibility with cleaning agents must be tested for these MOC in order to prevent damage or deterioration. There may be parts that are not submergible in water during cleaning, such as the feeder system housing (gear box – see Figure 17.2). A careful review of the equipment manufacturer's operation manual must be performed during the initial stages of cleaning method development to identify those parts that need alternate cleaning approaches.

Photos, drawings, or diagrams depicting how specific cleaning steps are to be performed is included in the cleaning procedure in order to minimize performance variability (refer to Figure 17.3). Disassembly of equipment and parts are also clearly described and depicted with photos or diagrams. Specific precautions, such as the identification of parts that should not be submerged in water, must be properly detailed in the cleaning instructions.

Figure 17.3: Pre-cleaning of a Tablet Compression Machine
Courtesy of Fette Compacting GmbH, Grabauer Str. 24, 21493 Schwarzenbek, www.fette-compacting.com. Image rights remain the property of Fette Compacting GmbH.



17.4 Manufacturing Process and Product Description

17.4.1 Manufacturing Process Considerations

Product A is a blue coated tablet (coating solution is blue, core tablet is white). The API for Product A is a white, free flowing dry powder. Product B is a white sugar-coated tablet (coating solution is white, core tablet is yellow). The API for Product B is a yellow, free flowing dry powder.

Both products use the same wet granulation process and utensils. During the dispensing process a stainless steel scoop is used. A small or medium sized spatula is used to remove the granulate blend from the sides of fluid bed dryer. The product granulate is transferred to the hopper of a rotary tablet compression machine for pressing into core tablets. The core tablets are transferred to the coating machine for enteric coating. The coating solution is prepared in a 25 L stainless pot and lid using a portable mixer, and the coating solution is pumped into the coating machine where it is sprayed onto the core tablets.

17.4.2 Residue Characteristics

Laboratory studies (beaker tests and coupon studies) were performed, PDE values were researched, and other relevant information was evaluated. The results are shown in Figure 17.1.

Table 17.1: Product Attributes

Attribute	Product A	Product B
Physical Characteristics: API	White free flowing dry powder Bulk density = 850 kg/m^3	Yellow free flowing dry powder Bulk density = 925 kg/m^3
Physical Characteristics: Coating Solution	Blue solution, easily dissolved in water at any temperature Low viscosity	White solution, highly soluble in water at any temperature Highly viscous and sticky
API Solubility in Water	Low solubility in water at room temperature Highly soluble in hot water (40°C)	Highly soluble in water at any temperature
Response to Alkaline Solution	Highly soluble at any temperature Commercial formula Minimum contact time = 5 min	Highly soluble at any temperature Commercial formula Minimum contact time = 3 min
Response to Sonication	Excellent response to loosen compacted API Sonication frequency = Medium Minimum duration = 6 min	Excellent response to loosen compacted API Sonication frequency = Low or Medium Minimum duration = 7 min
Scrubbing Time	Scoops = 1 min Spatulas = 1 min Mixing Blade = 2 min Machine Parts = 2 min Presoaking parts for 8 min reduces scrubbing time in half	Scoops = 1 min Spatulas = 1 min Mixing Blade = 2 min Machine Parts = 2 min Presoaking parts for 8 min reduces scrubbing time in half
PDE Value	95 µg/day	80 µg/day

It was confirmed that sonication is needed to dislodge all particles from the surface and allow them to dissolve rapidly during the wash steps. A soaking step prior to sonication reduced scrubbing times.

From a solubility perspective, Product A is the hardest to remove. High temperature was found to be optimum for dissolving all product API residues and coating solutions; however, water at hot temperatures present safety concerns when used for manual cleaning. A mild alkaline solution (10% commercial grade) to ensure solubility of soils was the preferred mode of action. A rinse step after using the alkaline solution is needed.

The PDE values for these products are high, which makes manual cleaning a viable approach. Residue results from swab samples coupon studies was always significantly lower than the safe cleaning limit based on PDE values.

The stainless steel 25 L vessel and lid are too heavy to move in and out of the sonicator system, and therefore were removed from this cleaning procedure, to be addressed in a different procedure. Based on the residue characterization, cleaning the vessel and lid with water at any temperature is sufficient to remove and dissolve residual coating solution. An additional cleaning procedure will be validated for cleaning vessels and lids.

The optimum conditions to safely remove the hardest-to-clean product consist of an initial rinse, sonication followed by a wash with alkaline solution at room temperature, followed by post wash rinse cycles and drying steps.

17.5 Recommended Cleaning Process for Tools and Small Parts

The initial recommended cleaning step sequence is:

1. Removal of gross residue via mechanical force (scrapping, wiping, brushing, and vacuuming)
2. Sonication step to loosen smaller particles adhered to surfaces hard to reach
3. Cleaning solution wash (alkaline)
4. Potable water rinse (to remove cleaning solution)
5. Purified Water final rinse
6. Drying
7. Visual Inspection
8. Storing cleaned items

These steps were further defined using specific parameters for cleaning operators to follow (see Table 17.2).

Table 17.2: Parameter Settings

Cycle Step	Action	Parameter Settings
Pre-Cleaning	Mechanical force (wiping, scraping, brushing, and vacuuming) Removes gross residue	Wipe all surfaces with non-shedding disposable wipes For tablet press surfaces – remove powder residue by vacuuming and brushing, followed by disassembling according to written procedure
Sonication	Loosens compacted residues from hard-to-reach surfaces via sonication cavitation forces at optimum frequency	Sonication frequency = Medium Flood all parts with water in the sonicator Potable water at room temperature Duration = minimum 10 min After sonication, scrub each part for minimum 2 min to loosen any remaining compacted soils.
Alkaline Solution Wash	Dissolves all product in washing solution	Replace potable water in sonication system with alkaline washing solution. Contact time = minimum 7 min

Table 17.2: Parameter Settings (continued)

Cycle Step	Action	Parameter Settings
Potable Water Rinse	Potable water rinse force	Rinse parts with potable water at 10 psig pressure through a standard cleaning nozzle. Manually rotate parts to ensure all sides are subjected to the rinse force. Duration = minimum 2 min
Final Rinse	Impingement and cascading force Purified Water, USP	Flood all parts with water: • Flow rate (velocity) = 1.5 m/s, 10 psig • Water temperature = Ambient • Time = 30 sec
Drying	Removes or evaporates water from part surfaces	Dry using clean, dry compressed air Wipe dry using clean, non-shedding disposable wipes Place dried items on a rack
Visual Inspection	Confirms visually clean criteria are met	Under appropriate lighting conditions, inspect each part to confirm visual cleanliness Do not use parts that fail to meet the criteria Return parts that fail visual inspection to dirty equipment area for investigation and cleaning
Ready for Storage	Cleaned, dried parts are protected and labeled	Individually place scoops and spatulas in plastic bags and seal, or cover in plastic wrap, and label Place tablet press parts into a plastic storage container with lid and label

17.5.1 Risk Assessment Considerations

The risk of cleaning failure was assessed via a discussion of what can go wrong during cleaning using a fishbone diagram as an assessment tool (refer to Figure 3.2), with the following results:

- Environmental hazards – No additional risks from the environment were identified. The temperature, room air changes, and humidity are controlled in the manufacturing suites, and no unexpected variations in these parameters are expected during the cleaning process.

- Methods hazards – The action of scrubbing a surface could introduce scratches on the surface of parts being cleaned and, with time, could develop into undesired surface conditions. Soils left too long on equipment after manufacturing can harden and become more difficult to remove consistently. Swabbing for residue levels is a well-established procedure performed by qualified quality personnel.

- Manpower hazards –Personnel may get tired if there are too many items to clean in one session. There is variability between operators in terms of techniques to clean and how consistent rinses and scrubbings are applied to the cleaning surfaces. Attention to scrubbing details or the ability to inspect surfaces may diminish with long cleaning process durations.

- Materials hazards – A 10% alkaline solution commercially available is used for cleaning. Water quality is controlled and monitored.

- Measurement hazards – Visual inspection of cleaning surfaces is conducted at the end, which requires clear instructions on how to perform the inspections and under which parameters. Other measurements such as time durations are tracked and documented with a digital clock located in the cleaning room.

- Machinery hazards – The sonicator has been qualified and water pressures used to perform manual rinsing are part of the preventive maintenance program. Hot water is not used, and there are no exposed moving parts that could represent a safety issue during cleaning.

Based on this assessment, the following risks were identified. Corresponding mitigation actions were proposed. See Table 17.3.

Table 17.3: Risks and Mitigation Actions

Risks	Probable Cause	Risk Mitigation Actions
Equipment is not cleaned consistently due to lack of clear procedure	Cleaning procedure not clear Inspection of cleaned surfaces not standardized Different tools may require different techniques for cleaning	• Review cleaning procedure steps and confirm sequence of operation, parameters, and any special cleaning technique for each part. • Modify SOPs, training programs, and documentation records to ensure process is clearly stated, followed, and documented.
Scratches can gradually form on surfaces to be cleaned, making the cleaning process harder or less effective	Too much force when scrubbing Unintended or undetected scratches and dents impacting cleaning effectiveness	• Introduce periodic inspection of cleaned items to look for changes in surface conditions. • Establish procedure to define criteria to replace or repair items after periodic inspections. • Define appropriate force when scrubbing and scraping surfaces, and include this information in training programs.
Product remaining on equipment cannot be removed consistently after DHT	Product can be hardened during DHT, making the removal of gross residues more difficult or inconsistent	• Introduce a "Pre-Cleaning" step as the last step in manufacturing to lower the amount of product on the equipment during DHT, which lowers the risk of not being able to clean the equipment after DHT.
Inconsistent visual inspections	Light levels during manual cleaning are not controlled Inspection techniques are not well defined	• Measure and control the type and intensity of light used during visual inspection of cleaned parts. • Train operators to verify that parameters for visual inspections are correct prior to conducting inspections.
Manual cleaning duration can become a distraction or an obstacle for consistent cleaning execution	Operators can become exhausted during manual cleaning activities	• Define a maximum time an operator can be assigned to manual cleaning activities and rotate personnel to ensure minimum distractions or to prevent personnel exhaustion during cleaning. • Minimize scrubbing time by incorporating a soaking step, which reduces scrubbing time by 50%.
Inconsistent manual cleaning	Variability of manual cleaning techniques between operators can impact cleaning process consistency	• Evaluate variability of cleaning results between operators and identify measures to increase cleaning process robustness (training, effectiveness checks, cleaning techniques). • Validate the optimum process that will clean all products to ensure effective and consistent cleaning results.
Critical cleaning parameters are not executed consistently	Critical parameters have not been identified among all cleaning parameters	• Identify the most important parameters to control in the cleaning process and highlight in procedures and training programs. Require these parameters to be documented in the cleaning record.

17.5.2 Final Recommendations for Cleaning Process

After completing the initial cleaning development activities and risks assessments, additional technical and procedural controls were incorporated into the recommended cleaning procedure. The initial process was modified accordingly:

- Procedures were modified to include specific instructions to clean each tool and small part.
- Maintenance procedures were updated to include inspection of tools and parts to confirm that surfaces are not damaged or compromised. A parts repair/replacement program was created.
- The training program was updated to minimize variability between operators, to include instructions on the appropriate application of scrubbing force, and to implement a visual inspection method that contains verification that an appropriate light source is available for inspection. Operators will be required to demonstrate successful execution of the cleaning process and will be qualified to perform visual inspections.
- Production schedules were improved to track operators assigned to manual cleaning processes and to rotate them periodically to prevent exhaustion of personnel. In addition, a soaking step was introduced in the cleaning process to reduce scrubbing time by 50%.
- Removal of gross product from product surfaces was moved from the cleaning process to the last steps of the manufacturing process to prevent hardening of product during DHT.

The recommended final steps sequence and relevant parameters are presented in Table 17.4.

Table 17.4: Parameters for the Cleaning Sequence

Step	Purpose	Parameters
Soaking	Wet the product adhered to equipment Reduce scrubbing time	*Soaking Time = minimum 10 min
Sonication	Loosen particles and compacted product from surfaces	*Sonication time = minimum 10 min Sonication frequency = Medium
Scrubbing	Mechanical action to remove product from surfaces	*Scrubbing duration = minimum 1 min
Alkaline Wash	Dissolve any remaining product from equipment surfaces	Solution concentration = 10% *Wash time = minimum 10 min
Potable Water Rinse	Remove alkaline solution	*Rinse time = minimum 2 min Rinse Pressure = 10 psig
Purified Water Final Rinse	Apply final rinse using Purified Water, USP	*Rinse Time = 30 secs Rinse Pressure = 10 psig
Drying	Remove water from tools and parts surfaces Reduce proliferation of microorganisms	Drying time = minimum 30 min Finish with dry wipe
Visual Inspection	Verify surfaces meet VC	*Light lumens = >200 lux at inspection level
Storage	Apply identification and protect tools and parts from environment to keep them cleaned until use	None
*Critical Parameters – Each facility needs to determine how to best capture critical parameters, either individually or as a group (e.g., total cleaning time for a piece or group of pieces). The adopted strategy needs to be clearly defined for consistent compliance.		

In line with GMP requirements, all cleaned items will be inspected prior to any re-assembly of parts and immediately prior to use. In the event that visual inspection of cleaned equipment reveals that the manual cleaning process was not properly performed, an investigation will be initiated and appropriate corrections taken (e.g., the personnel responsible may be required to undergo additional training and/or (re)qualification). A visual inspection failure will trigger an investigation to determine the root cause.

18 Appendix 7 – Case Study: Establishing Process Parameters for a Clean In Place Cleaning Process

This appendix demonstrates the application of CIP principles presented within this Guide. This case study is not meant to be a specification or a step-by-step process; instead it should be considered as an illustrative example of how cleaning principles can be adapted and applied to a hypothetical cleaning process or need.

18.1 Introduction

The purpose of this case study is to define the appropriate parameters for a CIP system to clean a 1,000 L formulation vessel after manufacturing various products. This formulation tank is not dedicated to manufacturing a single product but is a shared piece of equipment used in a multiproduct facility.

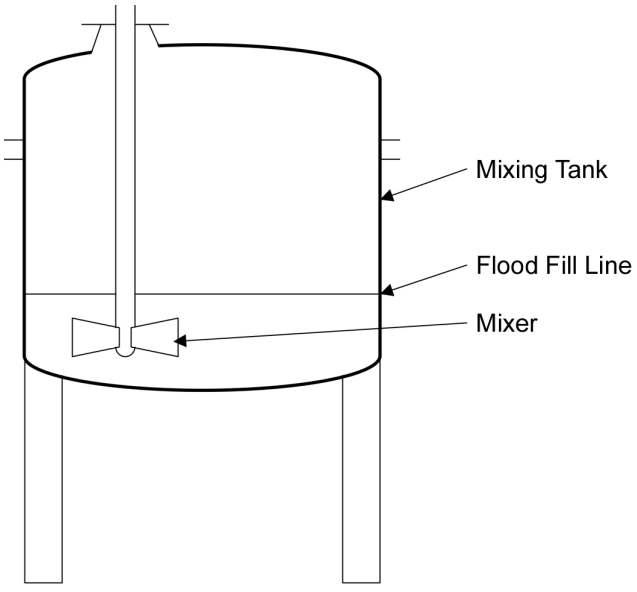
This appendix reinforces the information presented in Chapter 5 Cleaning Methodologies and Chapter 9 Equipment Issues and Challenges, providing examples of the practical application of that information. This case study assumes that the CIP system is available and has been properly designed, engineered, commissioned, and qualified.

18.2 System Description

The tank is used to formulate liquid product, mixing liquid-based or dry powder API, excipient(s), and diluent. This is a 1000 L cylindrical, dished (torisherical) bottomed 316 L SS tank with a mirror-polished finish (see Figure 18.1). The vessel is jacketed, capable of both heating and cooling, and fitted with a single blade SS propeller-type mixer, a pH probe, base mounted draining valve, 360° rotary CIP spray balls, and a hinged lid.

A maximum working volume is taken as 90% of the total tank volume (typical specification) (thus = 900 L). The minimum volume of the tank contents to assure that the mixer blade is fully covered is taken as 100 L. The minimum validated working volume of the vessel is 500 L (50% of the total tank volume).

Figure 18.1: Example Mixing Vessel



This vessel is connected to a 2-tank CIP system (one tank for alkaline cleaning solution and the other for acidic cleaning solution), which has automated input and outlet valves (2-pump system, supply pump and return pump, which allows rinse and wash solutions to be continuously removed from the vessel at the same rate as the supply). It also has in-line instruments measuring pH, volume and flow rate, temperature, time, and pressure. This integrated system provides excellent control over the TACT factors necessary for consistent execution of the cleaning process, that is, time, action (impingement force), chemical solution/concentration, and temperature.

18.3 Scenario 1 – Product A

18.3.1 Manufacturing Process Considerations

Product A consists of a liquid API, excipients, and Purified Water, USP as the process diluent. The API has a concentration of 50% in the final formulation and an ADE value of 5 mg/day. Product A can be manufactured in batch sizes of 500 L, 750 L, or 900 L.

Dissolving the API and excipients is performed at ambient temperature (no vessel heating required) with gentle agitation (10 rpm). The manufacturing process of Product A includes a final pH adjustment step to achieve the finished product's specified pH (8.0–10.0). At the final pH, the product is a clear liquid, but above pH 10.0 the liquid is red.

Product A is stored in this formulation vessel and then transferred directly from this vessel into the filling line hopper during filling operations. The maximum bulk holding time for Product A in this formulation vessel is 15 days.

18.3.2 Residue Characteristics

Laboratory studies (beaker tests and coupon studies) were performed on the API and Product A, and the following observations were made:

- The liquid API is highly soluble in water and easily dissolved in ambient temperature (25°C) water.
- The Product A final formulation is a clear, free-flowing liquid that is easily dissolved in water at any temperature – cold, ambient, or hot, has low viscosity, and very easy to clean.

For Product A, since the API and the final formulation are both very soluble in ambient temperature water, it is unnecessary to use a cleaning agent other than ambient temperature water. Potable water could be used for the initial rinse and wash cycles, but Purified Water, USP must be used in the final rinse cycle.

A flow rate velocity of 1.5 m/s provides adequate turbulent scrubbing action since the API and Product A are both very soluble; therefore it is not necessary to apply additional agitation by turning on the mixer during the cleaning cycle (i.e., the impingement and cascading flow of the water is adequate, as demonstrated during the CIP system qualification coverage studies). Since no cleaning solution is used during the wash phase (only water), there is no need to perform an intermediate rinse.

18.3.3 Proposed Cleaning Cycles for Product A

Table 18.1 presents the recommended cycles (refer to Section 5.2.7) for cleaning the vessel after manufacturing Product A.

Table 18.1: Product A Cleaning Cycles

Cycle	Action	Parameter Settings
Pre-CIP	Remove residual product	Drain all product liquid from tank
First Rinse	Potable water rinse with no recirculation, direct to drain	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 30 s
System Drain		3 min
Repeat for a total of 3 Rinse-Drain burst combinations.		
Wash Phase	Potable water used, may be recirculated	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 10 min Volume set to working volume
System Drain		3 min
Intermediate Rinse	Not required (since no cleaning agent was used)	N/A
System Drain	Not required (since no cleaning agent was used)	N/A
Final Rinse	Purified Water, USP	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 30 s
System Drain		3 min
One Rinse-Drain burst combination is performed.*		
Drying	Dry using clean, dry process air or heat from vessel jacket	15 min for air blowdown or 10 min for heating vessel jacket
*During cleaning cycle development, the TOC, conductivity, and pH of the Purified Water, USP was monitored by the CIP system instruments to confirm the number of final rinse cycles required to meet final cleaning criteria.		

18.4 Scenario 2 – Product B

18.4.1 Manufacturing Process Considerations

Product B consists of a dry powder API, excipients, and Purified Water, USP as the process diluent. The API has a concentration of 20% in the final formulation, with an ADE value of 10 µg/day. Product B can be manufactured in several different batch sizes – 500 L, 750 L, 900 L.

Dissolving the API and excipients is performed at ambient temperature (no vessel heating required) with moderate agitation (50 rpm). The manufacturing process of Product B includes a final pH adjustment step to achieve the finished product's specified pH (7.0–8.0).

Product B is stored in this formulation vessel and then transferred directly from this vessel into the filling line during filling operations. The maximum bulk holding time for Product B in this formulation vessel is 7 days.

18.4.2 Residue Characteristics

Laboratory studies (beaker tests and coupon studies) were performed on the API and Product B, and the following observations were made:

- The dry white-powder API is easily soluble/dissolved in ambient temperature water. Powder is dispersed in the formulation vessel empty volume during addition.

- The Product B final formulation is a slightly sticky, slightly viscous transparent liquid, light orange, that dissolves in ambient temperature water but is very easily dissolved in hot water (> 40°C). Due to the consistency of Product B (slightly sticky and slightly viscous) it was more difficult to clean than Product A when only water was used. Using an alkaline cleaning solution made the formulation residue much easier to clean with less effort.

For Product B, although the API is easily soluble in ambient temperature water, the finished formulation is less soluble in ambient water and more soluble and easier to clean using hot water (> 40°C). Use of an alkaline cleaning solution showed significant improvement over cleaning with water alone at ambient or hot temperatures; therefore, an alkaline solution was selected as the cleaning agent. Potable water could be used for the initial rinse and intermediate rinse cycles but Purified Water, USP must be used in the final rinse cycle.

A flow rate velocity of 1.5 m/s provides adequate turbulent scrubbing action; however, the viscous nature of the final formulation represents cleaning challenges for some areas of the mixing blade. Additional agitation is advised by turning on the mixer during the cleaning cycle and letting the vessel accumulate enough water to cover the blades (flood level line). The spray ball coverage, impingement, and cascading flow of the water was confirmed as adequate to cover all internal surfaces of the formulation tank, taking care of all surfaces to which the dispersed API powder may have adhered during formulation. When an alkaline cleaning solution is used during the wash phase, it is necessary to follow with an intermediate rinse (potable water, ambient temperature).

18.4.3 Proposed Cleaning Cycles for Product B

Table 18.2 presents the recommended cycles (refer to Section 5.2.7) for cleaning the vessel after manufacturing Product B.

Table 18.2: Product B Cleaning Cycles

Cycle	Action	Parameter Settings
Pre-CIP	Remove residual product	Drain all product liquid from tank
First Rinse	Potable water rinse with no recirculation, direct to drain	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 30 s
System Drain		3 min
Repeat for a total of 3 Rinse-Drain burst combinations.		
Wash Phase	Alkaline cleaning solution, recirculated	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 10 min Volume equal to flood line above agitator blades
System Drain		5 m
Intermediate Rinse	Potable water rinse, recirculated	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 10 min Volume equal to flood line above agitator blades
System Drain		5 min
Repeat for a total of 3 Rinse-Drain burst combinations.		
Final Rinse	Purified Water, USP	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 30 s
System Drain		3 min
One Rinse-Drain burst combination is performed.*		
Drying	Dry using clean, dry process air or heat from vessel jacket	15 min for air blowdown or 10 min for heating vessel jacket
*During cleaning cycle development, the TOC, conductivity, and pH of the Purified Water, USP was monitored by the CIP system instruments to confirm the number of final rinse cycles required to meet final cleaning criteria.		

18.5 Scenario 3 – Product C

18.5.1 Manufacturing Process Considerations

Product C consists of a dry powder API, excipients, and Purified Water, USP as the process diluent. The API has a concentration of 10% in the final formulation, with an ADE of 250 µg/day. Product C can be manufactured in several different batch sizes – 500 L, 750 L, 900 L.

Dissolving the API and excipients is performed at 40°C ± 5°C with an agitation speed of 70 rpm. The manufacturing process of Product C includes a final pH adjustment step to achieve the finished product's specified pH (7.0–8.0). At the final pH the product is a viscous emulsion.

Filling of Product C into its container-closure system should proceed directly after completion of the formulation process while the product is continuously heated and mixed (so that it stays homogenous and does not solidify or harden, and flows easily during filling). Product C is not to be stored in this formulation vessel for more than 24 hours and is transferred directly from this vessel into the filling line during filling operations.

18.5.2 Residue Characteristics

Laboratory studies (beaker tests and coupon studies) were performed on the API and Product C and the following observations were made:

- The dry white-powder API is soluble in water.
- The Product C final formulation is a viscous emulsion that leaves an oily, fatty residue on the equipment surface. Hot water (> 40°C) was considerably more effective than ambient temperature water. Water alone was not capable of removing the oily residue and an alkaline cleaning solution was required. Using an acidic cleaning solution following the alkaline solution improved the cleaning process.
- Product C is the hardest-to-clean product for this formulation tank.

For Product C, although the API is soluble in water, due to the formulation consistency (viscous, semi-solid/emulsion) an initial rinse with hot water (> 40°C) and use of an alkaline cleaning solution was required. Using an acidic cleaning solution showed marked improvement over cleaning with the alkali agent alone. Potable water could be used for the initial rinse and intermediate rinse cycles, but Purified Water, USP [46] must be used in the final rinse cycle.

Although a flow rate velocity of 1.5 m/s provides adequate turbulent scrubbing action, applying additional agitation by turning on the mixer during the cleaning cycle improved the cleaning process. The cleaning cycle includes letting the vessel accumulate enough water to cover the blades (flood level line) to ensure that the bottom surfaces of the blade are in contact with the cleaning solution.

18.5.3 Proposed Cleaning Cycles for Product C

Table 18.3 presents the recommended cycles (refer to Section 5.2.7) for cleaning the vessel after manufacturing Product C.

Table 18.3: Product C Cleaning Cycles

Cycle	Action	Parameter Settings
Pre-CIP	Remove residual product	Drain all product liquid from tank with instructions in the batch manufacturing record to immediately initiate the hot water initial rinse following removal of residual product.
First Rinse	Potable water rinse with no recirculation, direct to drain	Vessel mixer speed = 70 rpm Flow rate (velocity) set to 1.5 m/s Water temperature = 40°C Time set to 30 s
System Drain		3 min
Repeat for a total of 3 Rinse-Drain burst combinations.		
Alkali Wash Phase	Alkaline cleaning solution, recirculated	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 10 min Volume equal to the flood line above agitator blades
System Drain		5 min
Intermediate Rinse	Potable water rinse direct to drain	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 10 min Volume equal to the flood line above agitator blades
System Drain		5 min
Repeat for a total of 2 Rinse-Drain burst combinations.		
Acidic Wash Phase	Acidic cleaning solution, recirculated	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 5 min Volume equal to the flood line above agitator blades
System Drain		5 min

Table 18.3: Product C Cleaning Cycles (continued)

Cycle	Action	Parameter Settings
Intermediate Water Rinse	Potable water rinse, recirculated	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 10 min Volume equal to flood line above agitator blades
System Drain		5 min
Repeat for a total of 2 Rinse-Drain burst combinations.		
Final Rinse	Purified Water, USP	Flow rate (velocity) set to 1.5 m/s Water temperature = ambient Time set to 30 s
System Drain		3 min
One Rinse-Drain burst combination is performed.*		
Drying	Dry using clean, dry process air or heat from vessel jacket	15 min for air blowdown or 10 min for heating vessel jacket
*During cleaning cycle development, the TOC, conductivity, and pH of the Purified Water, USP [46] was monitored by the CIP system instruments to confirm the number of final rinse cycles required to meet final cleaning criteria.		

18.6 In-Process Monitoring and Visual Inspection

Throughout the cleaning process the interior of the vessel can be viewed from the manhole (looking through the glass with the assistance of the vessel light). Following the completion of the entire cleaning process a thorough visual inspection of the vessel's interior must be performed using a flashlight and with the hinged lid open to allow for full visibility of all surfaces. In addition, swab samples are taken from several justified locations of the formulation tank to confirm that the residue levels do not exceed the cleaning SL established for each product.

Once the development of the cleaning process is completed, the system is prepared for the execution of cleaning qualification runs.

18.7 Points to Consider

The residue characteristics greatly impact the cleaning process and the cycles required to successfully clean the equipment to the desired level on a consistent basis. As the examples of Product A, B, and C show, it may not be necessary to apply a single CIP "recipe" for all products manufactured on a given piece of equipment. However, using the most rigorous cleaning recipe for all products has several advantages: it minimizes the risk of using the wrong cleaning recipe; it lowers the risk of a cleaning failure since most products will be "overcleaned;" and it minimizes the cleaning validation effort and makes the program easier to explain to regulatory agencies. Not all products can be effectively cleaned with water only and not all products require the use of two cleaning agents during the wash phase (i.e., alkaline wash followed by acidic wash). It is necessary to effectively determine appropriate "groupings" for the cleaning regime (recipe) to be applied in order to minimize the potential risk of unsuccessful cleaning (refer to Section 5.6.1). The use of such groupings also allows increases in-process efficiency and minimizes waste (i.e., cleaning costs are reduced by only using hot water, cleaning agents, additional rinses, etc. for those products that actually require it).

19 Appendix 8 – Case Study: Application of Quality Risk Management Tools – Introduction of a New Product into an Existing Multiproduct Facility

ISPE Baseline® Guide: Volume 7 – Risk-Based Manufacture of Pharmaceutical Products [Risk-MaPP] (Second Edition) [3] provides several examples to illustrate the application of the *ISPE Risk-MaPP* tools and to demonstrate how a well conducted Quality Risk Management Plan (QRMP) can be used. Scenario 4 has been adapted to focus on cleaning issues and is included in this appendix; refer to the *ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3] for more information and examples.

Adapted from the *ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3] Scenario 4, Existing Facility with a Large Product Portfolio

19.1 Background Information

An existing facility is currently manufacturing multiple products using 9 different APIs. A new product (i.e., Anti-hypertensive2) needs to be introduced into the facility. The properties for the new product were assessed via product characterization studies, and the ADE information was researched and determined to be 400 µg/day. A table with the relevant information for all products was created to assess worst case conditions for cleaning validation. The products and APIs are shown in Table 19.1.

Table 19.1: Products to be Manufactured in the Facility

Product Code	API	API/Dose (mg)	Maximum Daily Dose (mg/day)	ADE (µg/day)	Batch Size (kg)	Batches/ Year	Product Contact Area (cm^2)	Process	Limit of Detection (µg/cm^2)
A101	Vitamin B3	500	2000	4200	450	6	464781	2	10.0
A102	Vitamin B3	750	2000	4200	240	6	464781	2	10.0
A103	Vitamin B3	1000	2000	4200	450	6	464781	2	10.0
B101	Anti-hypertensive1	2.5	10	25	4	18	1050646	3	3.0
B102	Anti-hypertensive1	5	10	25	7	18	1050646	3	3.0
C101	Anti-psychotic1	300	1800	830	240	10	392913	1	10.0
D101	Opioid	50	400	50	115	79	1178732	2	7.0
E101	Anti-epileptic	200	1600	250	160	19	519818	2	10.0
F101	Misc. Agent	150	600	9750	180	3	476619	2	25.0
F102	Misc. Agent	300	600	9750	300	14	479607	2	25.0
G101	Anti-cancer	50	150	170	8	6	320760	2	5.0
G102	Anti-cancer	150	150	170	18	3	333311	2	5.0
G103	Anti-cancer	50	150	170	65	4	459836	2	5.0
H101	Anti-psychotic2	50	800	280	75	8	418021	2	5.0
H102	Anti-psychotic2	100	800	280	160	21	492981	2	5.0
H103	Anti-psychotic2	200	800	280	300	8	492981	2	5.0
H104	Anti-psychotic2	300	800	280	300	11	492981	2	5.0
H105	Anti-psychotic2	400	800	280	300	9	492981	2	5.0
I101	Anti-psychotic3	150	450	1000	102	8	574846	3	15.0
I102	Anti-psychotic3	300	450	1000	102	8	607991	3	15.0
I103	Anti-psychotic3	150	450	1000	225	3	694867	3	15.0
I104	Anti-psychotic3	75	450	1000	30	3	653047	3	15.0
I105	Anti-psychotic3	100	450	1000	30	3	653047	3	15.0
J101	Anti-hypertensive2	50	1700	400	54	22	1050646	3	5.0
J102	Anti-hypertensive2	100	1700	400	54	4	1050646	3	5.0

Table 19.1: Products to be Manufactured in the Facility (continued)

Product Code	API	API/Dose (mg)	Maximum Daily Dose (mg/day)	ADE (µg/day)	Batch Size (kg)	Batches/ Year	Product Contact Area (cm^2)	Process	Limit of Detection (µg/cm^2)
J103	Anti-hypertensive2	50	1700	400	54	2	1083791	3	5.0
J104	Anti-hypertensive2	50	1700	400	9	18	1050646	3	5.0
J105	Anti-hypertensive2	25	1700	400	27	18	1105046	3	5.0
J106	Anti-hypertensive2	100	1700	400	150	51	203912	3	5.0
J107	Anti-hypertensive2	25	1700	400	100	32	570289	3	5.0
J108	Anti-hypertensive2	50	1700	400	149	46	177075	3	5.0

The processes used to manufacture the products are:

- Process 1: Sample, weigh, mill, granulate, mill, dry, mill, blend, compression, and pack (10 steps)
- Process 2: Sample, weigh, mill, granulate, mill, dry, mill, blend, compression, coat, and pack (11 steps)
- Process 3: Sample, weigh, mill, granulate, mill, dry, mill, blend, mill, blend, compression, mill, blend, compression, coat, and pack (16 steps)

All processes are fairly open (i.e., there are no containment devices or engineering controls used).

Cleanability studies were completed for the new product to be introduced taking into consideration which product was most difficult to clean. The new Anti-hypertensive2 product will use Process 3, which corresponds to the longest process ensuring a worst case from a production step perspective. The cleanability studies concluded that the new product would not constitute the most difficult product to clean, and therefore the existing cleaning procedure would be acceptable to clean after this product is manufactured. A change control assessment for validation considered both the hardest-to-clean and the lowest cleaning limit product. Based on the information provided, the change control assessment did not identify the need to revalidate the cleaning process; however, a verification run was requested and included in the new product introduction implementation plan. In addition, the analytical method used to measure the level of residues on cleaned surfaces will need to be verified as still appropriate for the full range of cleaning limits.

The cleaning limits are shown in Table 19.2.

Table 19.2: Cleaning Limits for the Products Manufactured

Product A (by API)	Product B (by API)	Cleaning Limit (µg/cm^2)	Product A (by API)	Product B (by API)	Cleaning Limit (µg/cm^2)
Vitamin B3	Anti-hypertensive1	3614.6	Misc. Agent	Vitamin B3	2517.3
Vitamin B3	Anti-psychotic1	1425.0	Misc. Agent	Anti-hypertensive1	8132.0
Vitamin B3	Opioid	2598.0	Misc. Agent	Anti-psychotic1	3309.0
Vitamin B3	Anti-epileptic	903.7	Misc. Agent	Opioid	5845.0
Vitamin B3	Misc. Agent	2711.0	Misc. Agent	Anti-epileptic	2033.0
Vitamin B3	Anti-cancer	698.0	Misc. Agent	Anti-cancer	1621.0
Vitamin B3	Anti-psychotic2	942.0	Misc. Agent	Anti-psychotic2	2187.0
Vitamin B3	Anti-psychotic3	603.0	Misc. Agent	Anti-psychotic3	1355.0
Vitamin B3	Anti-hypertensive2	47.8	Misc. Agent	Anti-hypertensive2	108.0
Anti-hypertensive1	Vitamin B3	6.5	Anti-cancer	Vitamin B3	44.4
Anti-hypertensive1	Anti-psychotic1	8.5	Anti-cancer	Anti-hypertensive1	147.9
Anti-hypertensive1	Opioid	6.8	Anti-cancer	Anti-psychotic1	57.7
Anti-hypertensive1	Anti-epileptic	4.8	Anti-cancer	Opioid	106.3
Anti-hypertensive1	Misc. Agent	15.8	Anti-cancer	Anti-epileptic	37.0
Anti-hypertensive1	Anti-cancer	4.2	Anti-cancer	Misc. Agent	110.9
Anti-hypertensive1	Anti-psychotic2	5.6	Anti-cancer	Anti-psychotic2	38.1
Anti-hypertensive1	Anti-psychotic3	2.6	Anti-cancer	Anti-psychotic3	24.7
Anti-hypertensive1	Anti-hypertensive2	0.1*	Anti-cancer	Anti-hypertensive2	2.0*

Table 19.2: Cleaning Limits for the Products Manufactured (continued)

Product A (by API)	Product B (by API)	Cleaning Limit (µg/cm²)	Product A (by API)	Product B (by API)	Cleaning Limit (µg/cm²)
Anti-psychotic1	Vitamin B3	253.5	Anti-psychotic2	Vitamin B3	72.0
Anti-psychotic1	Anti-hypertensive1	845.0	Anti-psychotic2	Anti-hypertensive1	227.0
Anti-psychotic1	Opioid	607.3	Anti-psychotic2	Anti-psychotic1	95.0
Anti-psychotic1	Anti-epileptic	211.2	Anti-psychotic2	Opioid	163.0
Anti-psychotic1	Misc. Agent	633.7	Anti-psychotic2	Anti-epileptic	57.0
Anti-psychotic1	Anti-cancer	138.0	Anti-psychotic2	Misc. Agent	176.0
Antipsychotic1	Anti-psychotic2	198.0	Anti-psychotic2	Anti-cancer	47.0
Antipsychotic1	Anti-psychotic3	140.9	Anti-psychotic2	Anti-psychotic3	38.0
Antipsychotic1	Anti-hypertensive2	11.0	Anti-psychotic2	Anti-hypertensive2	3.0
Opioid	Vitamin B3	6000000	Anti-psychotic3	Vitamin B3	258.2
Opioid	Anti-hypertensive1	20000000	Anti-psychotic3	Anti-hypertensive1	575.6
Opioid	Anti-psychotic1	6666666.7	Anti-psychotic3	Antipsychotic1	339.3
Opioid	Anti-epileptic	5000000	Anti-psychotic3	Opioid	413.7
Opioid	Misc. Agent	15000000	Anti-psychotic3	Anti-epileptic	192.4
Opioid	Anti-cancer	2666666.668	Anti-psychotic3	Misc. Agent	629.4
Opioid	Anti-psychotic2	4687500	Anti-psychotic3	Anti-cancer	166.3
Opioid	Anti-psychotic3	3333333.3	Anti-psychotic3	Anti-psychotic2	224.3
Opioid	Anti-hypertensive2	264705.9	Anti-psychotic3	Anti-hypertensive2	7.6
Anti-epileptic	Vitamin B3	64.5	Anti-hypertensive2	Vitamin B3	103.3
Anti-epileptic	Anti-hpertensive1	192.4	Anti-hypertensive2	Anti-hypertensive1	152.3
Anti-epileptic	Antipsychotic1	84.8	Anti-hypertensive2	Antipsychotic1	135.7
Anti-epileptic	Opioid	138.3	Anti-hypertensive2	Opioid	106.0
Anti-epileptic	Misc. Agent	157.4	Anti-hypertensive2	Anti-epileptic	77
Anti-epileptic	Anti-cancer	41.6	Anti-hypertensive2	Misc. Agent	251.8
Anti-epileptic	Antipsychotic2	56.1	Anti-hypertensive2	Anti-cancer	66.5
Anti-epileptic	Antipsychotic3	32.1	Anti-hypertensive2	Antipsychotic2	89.7
Anti-epileptic	Anti-hypertensive2	2.6	Anti-hypertensive2	Antipsychotic3	40.8

The updated cleaning matrix revealed that the lowest limit to satisfy all combinations of product manufacturing corresponds to a level of 0.1 µg/cm² when cleaning after manufacturing Anti-hypertensive1 prior to manufacturing Anti-hypertensive2. This represents the lowest cleaning limit of all product combinations and requires the facility to apply it as the cleaning limit that can cover all products in the absence of additional engineering or process controls. The cleaning limit of 0.1 µg/cm² will be used as the acceptance criteria for validation as well as for the routine verification/monitoring program.[26]

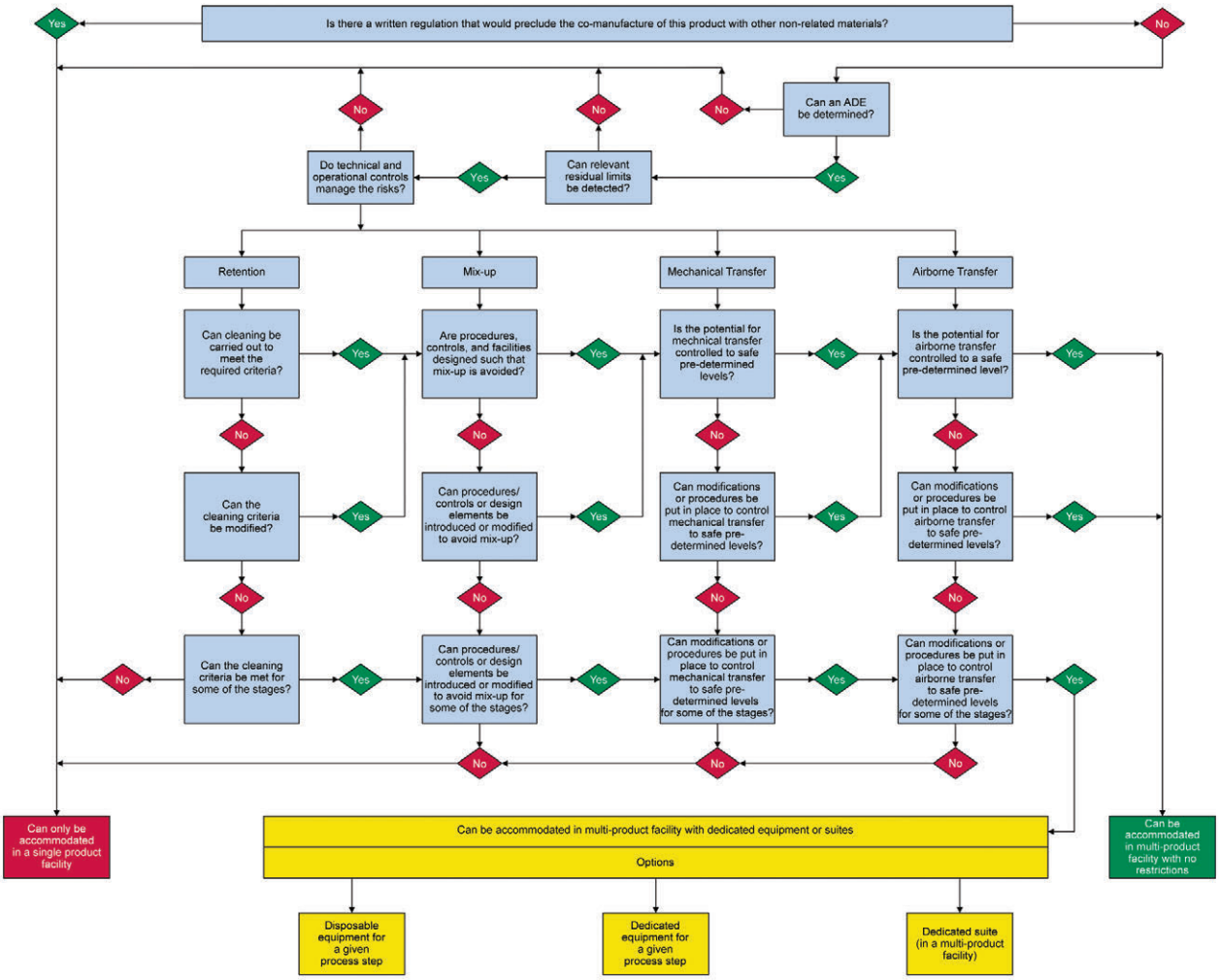
The cleaning procedures are all manually based with only a visual inspection by the operator and a supervisor to verify the equipment is cleaned to the limits (0.1 µg/cm²).

19.2 Risk Assessment

The logic diagram in the *ISPE Baseline® Guide: Risk-MaPP (Second Edition)* Section 14.6 [3] was used to walk through the risk assessment steps. The logic diagram presents a series of questions to guide the user toward one of the following options when assessing risks for cross-contamination in a facility: use dedicated facilities, use multiproduct facilities with dedicated suites and/or equipment, or use multiproduct facilities without restrictions. See Figure 19.1 for a portion of the logic diagram. *Refer to ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3] for a full description of the diagram and its applicability.

[26] Applying the lowest limit for all products represents a very conservative approach given that higher limits are allowed for other products in the matrix. The facility has a single cleaning product matrix and has not implemented grouping strategies for cleaning purposes. Product grouping options could be a viable strategy to alleviate the cleaning effort and de-risk potential failures due to applying the lowest cleaning limit for all products instead of for selected groups of products. Another optimization strategy to consider to manufacture the new product Anti-hypertensive2 in a dedicated facility or on dedicated equipment. This would increase the lowest overall cleaning limit for all of the other products to 2.6 µg/cm², making cleaning process qualification easier to complete successfully.

Figure 19.1: GMP/Regulatory Factors



Risk Analysis

A review of internal quality system documentation and regulatory filings did not identify a requirement to manufacture these types of products in a dedicated facility. The team completed risk analysis of the four modes of cross-contamination; mix-up, retention, mechanical transfer, and airborne transfer to be able to answer the remaining questions on the logic diagram in *ISPE Baseline® Guide: Risk-MaPP (Second Edition)* Section 14.6 [3]. Out of the four modes of cross-contamination, cleaning is most relevant to the Retention mode. The logic diagram (see Figure 19.1) includes the following questions to assess the cross-contamination risks via Retention mode:

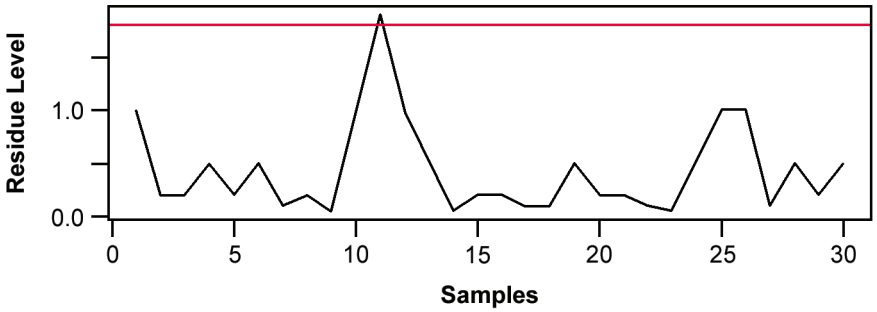
- Can cleaning be carried out to meet the required criteria?
- Can the cleaning criteria be modified?
- Can the cleaning criteria be met for some of the stages?

A negative answer to all of these questions would result in a recommendation to manufacture the product in a dedicated facility.

Analysis of Cleaning Data

Historical cleaning results indicate that one cleaning event failed to meet the acceptance criteria over 30 cleaning events (see Figure 19.2). As a result of investigations the root cause of the failure was determined to be that the procedure was not followed. Corrective action (additional training) was taken. No additional failures have been observed since the corrective action was implemented.

Figure 19.2: Process Control Testing



Determining Worst-Case for Overall Cross-Contamination Risk Assessment (Highest Risk Compound)

A worst-case approach is used to determine which combination of products will be used in the risk assessments. To determine the high-risk products combine the hazard properties (ADE, % API, volumes manufactured, and frequency of manufacture) and the process properties (process, equipment used, openness of equipment) by API.

Based on above, the APIs are ranked as shown in Table 19.3.

Table 19.3: Risk Ranking of APIs Manufactured

API	Maximum Daily Dose (mg/day)	ADE (µg/day)	Largest Batch Size (kg)	Batches/ Year	Process	Risk Ranking
Vitamin B3	2000	4200	450	18	2	6
Anti-hypertensive1	10	25	7	36	3	10
Anti-psychotic1	1800	830	240	10	1	4
Opioid	400	50	115	79	2	1
Anti-epileptic	1600	250	160	19	2	3
Misc Agent	600	9750	300	17	2	8
Anti-cancer	150	170	65	13	2	9
Anti-psychotic2	800	280	300	57	2	2
Anti-psychotic3	450	1000	225	25	3	7
Anti-hypertensive2	1700	400	150	193	3	5

Factors that contribute to make the opioid the high-risk product are:

1. The ADE is fairly low compared to the other compounds
2. The product is manufactured fairly regularly
3. The maximum daily dose is in the middle range for the products
4. The process does not have a huge impact as most of the products are using the same process and those using more processing steps have greater batch sizes, ADEs, and MDDs

Based on the above risk ranking, the risk analysis used the opioid as the worst-case product for assessing cross-contamination risks (not to be confused with worst-case product for cleaning validation).

Note: that all the process steps are covered in the opioid's process so no additional products need to be analyzed.

Fishbone Analysis (Partial)

The team brainstormed ways in which the four modes could occur. The results are documented in Figure 19.3. An FMEA was conducted, as shown in Table 19.4.

Figure 19.3: Fishbone Analysis (example)

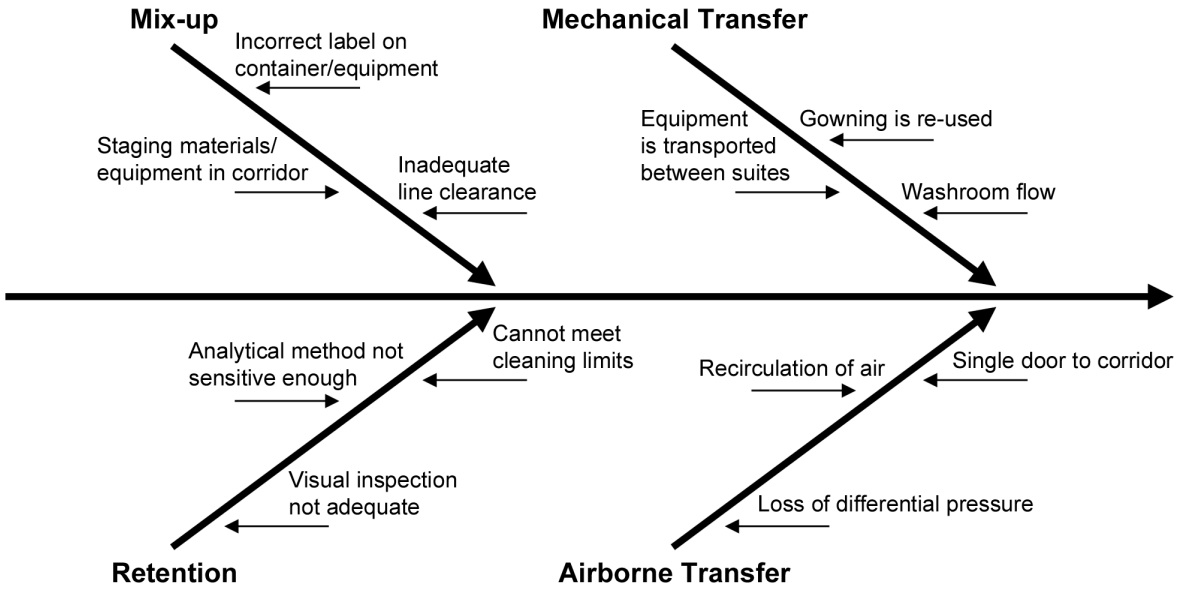


Table 19.4: FMEA Analysis (partial)

Facility	Process Step	Potential Failure	Potential Effect(s) of Failure	Severity	Potential Causes	Occurrence	Current Controls	Detection	Risk Priority Number (RPN)
OSD	Receiving	Wrong label	Mix-up	10	Human Error	3	2-person sign off	7	210
OSD	Compounding	Wrong Materials brought to room	Mix-up	10	Human Error – equipment staged in corridor	5	2-person sign off	7	350
OSD	Compounding	Wrong Materials brought to room	Mix-up	10	Human Error – wrong label	3	2-person sign off	7	210
OSD	Compounding	Equipment not Clean	Retention	10	Visual not adequate	7	2-person sign off	10	700
OSD	Compounding	Equipment not Clean	Retention	10	Human Error – did not follow procedure	3	2-person sign off	7	210
OSD	Compounding	Gown not changed when moving from API to API	Mechanical transfer	5	Inadequate procedure	7	Procedure	10	350
OSD	Compounding	Residue on materials and equipment brought into room	Mechanical transfer	3	Inadequate procedure	7	Procedure	10	210

Table 19.4: FMEA Analysis (partial) (continued)

Facility	Process Step	Potential Failure	Potential Effect(s) of Failure	Severity	Potential Causes	Occurrence	Current Controls	Detection	Risk Priority Number (RPN)
OSD	Compounding	Loss of pressure differential	Airborne transfer	5	Single door to corridor – door open	5	Manually check magnehelic at start of shift	7	175
OSD	Compounding	Airborne residue	Airborne transfer	5	Inadequate filtration/ recir-culated air supply	10	Facility design	1	50
OSD	Compression	Foreign tablets	Mix-up	10	Human Error – line clearance	3	2-person sign off	7	210
OSD	Compression	Equipment not Clean	Retention	10	Visual not adequate	7	2-person sign off	10	700
OSD	Compression	Equipment not Clean	Retention	10	Human Error – did not follow procedure	3	2-person sign off	7	210
OSD	Compression	Residue on materials and equipment brought into room	Mechanical transfer	7	Inadequate procedure	10	Procedure	7	490
OSD	Compression	Gown not changed when moving from API to API	Mechanical transfer	7	Inadequate pro-cedure	10	Procedure	10	700
OSD	Compression	Loss of pressure differential	Airborne transfer	7	Single door to corridor – door open	5	Manually check magnehelic at start of shift	7	245
OSD	Compression	Airborne residue	Airborne transfer	7	Inadequate filtration/ recirculated air supply	10	Facility design	1	70

Risk Evaluation

The corporate guidance on risk management states the following actions for RPN ranges:

1. Acceptable Risk: RPNs less than 125
2. Risk Reduction: should be applied for RPNs 125 to 343
3. Unacceptable risk: cease until risk is reduced for RPNs above[27]

Risk Control

Activities must cease until risk reduction is implemented for the following items:

1. Mix-up: staging in the corridor

[27] A core element of the FMEA tool is to use risk scores. Scoring is arbitrary and as such extreme caution should be applied when scoring is used as a decision-making process for risk mitigation. Approaches to set risk scores (e.g., RPN) should be defined by qualified personnel and supported by a sound, consistent, and thoroughly documented process.

2. Retention: visual not adequate to meet acceptance criteria

3. Mechanical transfer: gowns not changed when changing API

4. Mechanical transfer: residue on materials and equipment brought into/out of process room

Activities can continue but risk reduction should be implemented for the following items:

1. Mix-up: wrong label on container

2. Retention: equipment not clean/did not follow procedure

3. Airborne transfer: loss of pressure differential

Risk Communication

Immediate notification was provided to senior management on the unacceptable risk areas. Weekly updates on progress will be provided to senior management and operations until the risk is reduced to allow for regular production to continue.

Change control will be used to alert of possible changes that could alter the assessments, conclusions, and risk profile of the facility.

CAPA will be used to manage all risk reduction activities.

Training on how to manage the risk of cross-contamination will be provided to all staff involved in the manufacture and/or packing of the products.

Risk Review

The risk assessment will be reviewed:

1. When products are added or removed from the facility portfolio

2. When facilities, equipment and/or processes change or are added

3. When there is a change to a product's properties (MDD, ADE, etc.)

4. At least annually

Risk Summary

There are several items that must be remediated in order to resume production. These are staging of materials in the corridor, visual detection for clean equipment is not always adequate based on the acceptance criteria, gown changes when changing API, and residue escaping rooms on materials and equipment.

The team is preparing remediation plans and will update senior management and operations on a weekly basis until the risk is reduced to allow production to resume.

20 Appendix 9 – References

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21 Appendix 10 – Glossary

21.1 Acronyms and Abbreviations

ADE	Acceptable Daily Exposure
API	Active Pharmaceutical Ingredient
APIC	Active Pharmaceutical Ingredients Committee
ARL	Acceptable Residue Limit
ASME	American Society of Mechanical Engineers
ASTM	American Society for Testing and Materials
ATCC®	American Type Culture Collection
BET	Bacterial Endotoxin Test
BPE	Bioprocessing Equipment (ASME National Standard)
CAD	Computer-aided Design
CAPA	Corrective and Preventive Action
CCV	Continued Cleaning Verification
CDA	Clean, Dry Air
CE	Capillary Electrophoresis
CFU	Colony Forming Unit
CGMP	Current Good Manufacturing Practice
CHMP	Committee on Human Medicinal Products (United Kingdom)
CHT	Clean Hold Time
CIP	Clean in Place
COP	Clean Out of Place
CPP	Critical Process Parameter
CPV	Continued Process Verification
CQA	Critical Quality Attribute
CV	Cleaning Validation
CVP	Cleaning Validation Plan
CVMP	Cleaning Validation Master Plan
D/E	Dey/Engley
DHT	Dirty Hold Time
DIN	Deutsches Institut für Normung eV (German Institute for Standardization)
DL	Detection Limit
DOE	Design of Experiment

DP	Drug Product
DS	Drug Substance
DV	Dilution Volume
EDTA	Ethylenediamine Tetraacetic Acid
EHEDG	European Hygienic Engineering & Design Group
EIA	Enzyme Immunoassay
ELISA	Enzyme-linked Immunosorbent Assay
EMA	European Medicines Agency
EPA	Environmental Protection Agency (US)
EQ	Equipment
EU	Endotoxin Unit
EU	European Union
FDA	Food and Drug Administration (US)
FMEA	Failure Mode Effects Analysis
GC	Gas Chromatography
GMP	Good Manufacturing Practice
GxP	Good "x" Practice
HACCP	Hazard Analysis and Critical Control Point
HBEL	Health-based Exposure Limit
HMI	Human-Machine Interface
HTP	Human Therapeutic Protein
HVAC	Heating, Ventilation, and Air Conditioning
IFA	Inactive Fragments of Product A
IFA	Inactive Fragments of Product B
IMP	Investigational Medicinal Product
IP	Intermediate Precision
IPA	Isopropyl Alcohol
IR	Infrared
ISO	International Organization for Standardization
IV	Intravenous
LAL	Limulus Amebocyte Lysate
LPS	Lipopolysaccharide
LOD	Limit of Detection
LOQ	Limit of Quantitation

LRW	LAL Reagent Water
MACO (MAC)	Maximum Allowable Carryover
MBS	Minimum Batch Size
MDD	Maximum Daily Dose
MHRA	Medicines and Healthcare products Regulatory Agency (UK)
MOC	Material of Construction
MRA	Mutual Recognition Agreement
MS	Mass Spectrometry
MSC	Maximum Safe Carryover
MW	Molecular Weight
NMT	Not More Than
NOAEL	No-Observed-Adverse-Effect-Levels
NPSH	Net Positive Suction Head
OSD	Oral Solid Dosage
PBST	Phosphate-Buffered Saline with 0.04% Tween® 80
PCO	Product Changeover Procedures
PDA	Parenteral Drug Association
PDE	Permitted Daily Exposure
pFMEA	Process Failure Mode Effect Analysis
PIC/S	Pharmaceutical Inspection Co-operation Scheme
PPQ	Process Performance Qualification
PQ	Performance Qualification
PR	Periodic Review
PSIA	Product Specific Immunoassay
PTFE	Polytetrafluoroethylene
PV	Process Validation
PW	Purified Water
QA	Quality Assurance
QC	Quality Control
QL	Quantitation Limit
QRM	Quality Risk Management
QRMP	Quality Risk Management Plan
q.s.	quantum sufficit
RCF	(Bioburden) Recovery Correction Factor

RF	Recovery Factor
RPN	Risk Priority Number
RS	Reference Standard
RSD	Relative Standard Deviation
SCADA	Supervisory Control and Data Acquisition
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
SE-HPLC	Size Exclusion High Pressure Liquid Chromatography
SIP	Steam in Place
SL	Safety Limit
SME	Subject Matter Expert
SOP	Standard Operating Procedure
SPC	Statistical Process Control
SS	Stainless Steel
SSA	Sample Surface Area
STDD	Standard Therapeutic Daily Dose
SUT	Single-Use Technology
TACT	Time, Action, Chemical, and Temperature
TBD	To Be Determined
TLC	Thin Layer Chromatography
TOC	Total Organic Carbon
TRC	Test Result Correction
TS	Test Solution
TSA	Trypticase Soy Agar
TSE	Transmissible Spongiform Encephalopathy
TTC	Threshold of Toxicological Concern
UPLC	Ultra-Performance Liquid Chromatography
USP	United States Pharmacopeia
VC	Visually Clean
VMP	Validation Master Plan
VRL	Visible Residue Limit
WFI	Water for Injection
WHO	World Health Organisation

21.2 Definitions

Acceptable Daily Exposure (ADE) (*ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3])

A dose that is unlikely to cause an adverse effect if an individual is exposed, by any route, at or below this dose every day for a lifetime. By definition the ADE is intended to be protective of all subpopulations and by all routes of administration. Establishment of the ADE value is an important first step in a risk assessment and the inherent assumptions should be consistent with how it is applied within the product mix in a given facility.

Note: When calculating the cleaning threshold values, the terms PDE, ADE, and HBEL are used interchangeably in this Guide.

Acceptance Limit

Maximum acceptable residue that is allowed to be carried over and considered safe.

Active Ingredient

Any component of a drug product intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of humans or other animals. Active ingredients include those components of the product that may undergo chemical change during the manufacture of the drug product and be present in the drug product in a modified form intended to furnish the specified activity or effect.

Active Pharmaceutical Ingredient (API) (FDA [20])

Any substance or mixture of substances intended to be used in the manufacture of a drug (medicinal) product and that, when used in the production of a drug, becomes an active ingredient of the drug product. Such substances are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease or to affect the structure or function of the body.

Accuracy (ICH Q2 [107])

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.
This is sometimes termed trueness.

Action Limit (also known as **Action Level**) (ISO 14698 [10])

A parameter set by the user that, when exceeded, requires immediate intervention, including investigation of cause, and corrective action.

Agitated Immersion

A cleaning system in which the manufacturing equipment is placed in the cleaning solution, and the cleaning solution is agitated, usually with the existing agitation equipment in that equipment.

Agitation

The mixing or movement of the cleaning solution in the equipment. Agitation may occur from flow of the cleaning solution, or it may be due to mixers or impellers.

Alert Level (also known as **Alert Limit**) (ISO 14698 [10])

A parameter set by the user that, when exceeded, gives an early warning of a drift from normal operational conditions, and should result in increased attention or corrective action.

Bioburden (ISO 17665 [121])

Population of viable microorganisms on and/or in a product and/or sterile barrier system.

Blank

Test sample with a background value that may be subtracted from an experimental value to determine the "true" value

Campaign

Multiple lots or batches of the same product manufactured serially in the same equipment over a period of time.

Clean Hold Time (CHT)

Time from the end of the cleaning process until the equipment is used again.

Clean in Place (CIP)

Internally cleaning a piece of equipment without relocation or disassembly. The cleaning is normally done using solvent, chemical or detergent or a combination, with purified water rinse.

Cleanability

The ability to reduce a residue of a product to an acceptable level

Cleaning Agent

Chemical agent or solution used for cleaning. May be aqueous or solvent-based.

Cleaning Development

Work done before the validation protocol to establish a cleaning SOP. May involve lab, pilot-scale, as well as full process testing.

Cleaning Limit – see **Safety Limit**

Cleaning Procedure

An approved written procedure with sufficient detail to consistently produce an acceptable level of cleanliness in a piece of equipment.

Cleaning Process

The activity of cleaning that is used to remove materials introduced into equipment as part of the manufacturing or packaging process stream. These materials may include: formulation ingredients – API and excipients.

Cleaning Process Performance Qualification

Documented evidence with a high degree of certainty that the cleaning process will consistently meet the predetermined acceptance limits.

Cleaning Validation (EU [4])

Cleaning validation is documented evidence that an approved cleaning procedure will reproducibly remove the previous product or cleaning agents used in the equipment below the scientifically set maximum allowable carryover level.

Cleaning Verification (EU [4])

The gathering of evidence through chemical analysis after each batch/campaign to show that the residues of the previous product or cleaning agents have been reduced below the scientifically set maximum allowable carryover level.

Clean Out of Place (COP)

An automated or semiautomated procedure in which the process equipment is disassembled and its components are placed into an agitated fluid bath of cleaning solutions. Baskets may be utilized to hold and wash smaller parts such as gaskets, clamps, valve bodies, PD pump rotors, etc. The cleaning is normally done with a proprietary acid or caustic-based detergent, or a combination of both, with a compendial water rinse.

Contaminant

Drug substance, excipient, degradant, processing aid, cleaning agent, or foreign matter that, at a high enough level remaining after cleaning, may potentially contaminate the equipment surfaces or the next product.

Continued Process Verification (CPV) (FDA [5])

Assuring that during routine production the process remains in a state of control.

Control Strategy (EU [4])

A planned set of controls derived from current product and process understanding that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications and the associated methods and frequency of monitoring and control.

Coupon

Small model surface used for either laboratory testing of cleaning performance, or for swab or rinse recovery studies.

Critical Cleaning Parameters

Measured cleaning process attributes that are critical when variation over the range of its common use can cause variability that leads to an unacceptable CQA (e.g., cleaning time).

Critical Quality Attribute (CQA) (ICH Q8 [122])

A physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

Dedicated Equipment

Equipment only used for the manufacture of one product or one related product line.

Detection Limit (DL) (ICH Q2 [107])

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. (See also Limit of Detection.)

Detergent

A type of cleaning agent, usually aqueous based and utilizing surfactants.

Direct Product Contact Surfaces

That part of the manufacturing or packaging equipment that is in direct contact with the product during its normal flow through the unit.

Dirty Hold Time (DHT)

Time from the end of product manufacture or packaging until the beginning of the cleaning process

Drug Product (DP) (FDA [20])

A finished dosage form, for example, a tablet, capsule or solution that contains an active pharmaceutical ingredient, generally, but not necessarily, in association with inactive ingredients.

Drug Substance (DS) – see **Active Pharmaceutical Ingredient**

Endotoxin

Pyrogens from certain Gram negative bacteria. Generally highly toxic Lipopolysaccharide-protein complexes (fat, linked sugars, and protein) from cell walls. A marker for these bacteria with a reputation for persistent contamination because they tend to adhere to surfaces.

Equipment Train

Series of individual pieces of equipment linked together for a given process. May be cleaned individually or as a process train.

Finish *(surface)*

Degree of roughness or smoothness of a surface.

Grouping Strategy

Validation strategy for multiproduct equipment in which Performance Qualification (PQ) runs are performed on a defined group of equipment (grouping criteria justified by equipment similarities and same cleaning procedures), using a representative product (usually the most difficult to clean), and that performance is considered representative of the cleaning of all products and equipment within that defined group.

Highly Hazardous Compounds (*ISPE Baseline® Guide: Risk-MaPP (Second Edition)* [3])

Compounds with low ADE/PDE values, for example, ≤ 10 µg/day.

Health-Based Exposure Limits[28] (HBEL) (EMA [11])

A daily dose or a substance below which no adverse effects are anticipated, by any route, even if exposure occurs for a lifetime. Derived from a structured scientific evaluation of relevant data.

Impingement

Process of a cleaning solution striking a surface. Impingement usually occurs in a spray process, and helps dislodge soils from surfaces.

Indirect Product Contact Surfaces

Equipment surfaces shared among multiple products yet are not intentionally subjected to the processing pathway, for example, mechanical spaces of tablet presses and encapsulates, fluid bed dryer filter housing, tray drying oven cavities, and lyophilization cavities.

Interference

Something in an analyzed sample which causes analytical results for the target analyte to less precise, less accurate, or just less applicable.

Intermediate Precision (IP) (ICH Q2 [107])

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Limit of Detection (LOD)

Lowest level of analyte that can be detected, but not necessarily quantified. (See also Detection Limit.)

Limit of Quantitation (LOQ)

Lowest level of analyte that can be reliably measured with acceptable accuracy and precision. (See also Quantitation Limit.)

Linearity (ICH Q2 [107])

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Margin of Safety (ASTM E3106 [33])

The difference between the cleaning acceptance limit (based on ADE) and the process residue data.

[28] Establishing a HBEL involves the identification of hazard conditions (toxicity), evaluating the therapeutic or adverse effects, determining NOAEL (mg/kg/day), establishing a PDE or ADE, and calculating a MACO [11].

Maximum Allowable Carryover (MACO) (also known as **MAC**) (ASTM E3219 [12])

Calculated quantity of residue from a previous product when carried over into a different product that can represent potential harm to the patient.

With the introduction of safe cleaning limits based on HBELs, the MACO term should be considered a Maximum Safe Carryover (MSC), which is the maximum amount of carryover of a residual process residue (API, cleaning agent, degradant, and so forth) into the next product manufactured without presenting an appreciable health risk to patients.

Multi-Use Equipment

Equipment used for the manufacture of multiple products

Neutralization

Process of changing the pH of a used aqueous cleaning solution to the "neutral" range of approximately 6–10 so it can be discharged into a waste treatment system.

Nonhazardous Compounds

Compounds with high ADE/PDE values, for example, ≥ 100 µg/day.

Non-Product Contact Surface

Surfaces that are not in contact with process or product flow paths. Non-product contact surfaces may represent a risk of contamination for other process surfaces, and should be addressed as part of the overall cross-contamination strategy for the system or facility.

Permitted Daily Exposure (PDE) (EMA [11])

The PDE represents a substance-specific dose that is unlikely to cause an adverse effect if an individual is exposed at or below this dose every day for a lifetime.

Note: When calculating the cleaning threshold values, the terms PDE, ADE, and HBEL are used interchangeably in this Guide.

Precision (ICH Q2 [107])

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Process Capability

The level to which the cleaning process can consistently remove residues.

Process Contact Surface (ISPE [123])

Surfaces that, under design operating conditions, are in contact with or have the potential to contact raw materials, in-process materials, APIs, clean utilities (e.g., WFI, CIP, pure steam, process gases), or components (e.g., stoppers), and where there is a potential for the surface to affect product safety, quality, identity, strength, or purity.

Process Qualification

Part of validation that documents cleaning performance of the SOP.

Product Contact Surface (ISPE [123])

Process contact surfaces that are in contact with product, where product is defined by the owner/user. Examples of product contact surfaces may include the interior surfaces of bioreactors, transfer tubing, chromatography columns, vessels, and recirculating segments of CIP systems.

Purified Water (USP [46])

Water rendered suitable for pharmaceutical purposes by using unit operations that include deionization, distillation, ion exchange, reverse osmosis, filtration, or other suitable purification procedures. It meets rigid specifications for chemical purity, the requirements of the Federal Environmental Protection Agency (EPA) with respect to drinking water, and it contains no added substances. Cannot be used as raw material for parenterals. Common uses are: a rinse for equipment, vials, and ampoules, and as makeup for cosmetics, bulk chemicals, and oral products. For FDA acceptance, Purified Water must contain less than 0.5 mg/L of TOC (Total Organic Carbon), and less than 100 CFU.

Purified Water (Ph. Eur. [109])

Water for the preparation of medicinal products other than those that require the use of water which is sterile and/or apyrogenic. Purified Water which satisfies the test for endotoxins may be used in the manufacture of dialysis solutions. Purified Water is prepared by distillation, by ion exchange or by any other suitable method that complies with the regulations on water intended for human consumption laid down by the competent authority.

Quantitation Limit (ICH Q2 [107])

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. (Also known as Limit of Quantitation.)

Range (ICH Q2 [107])

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Recirculation

CIP cleaning process, or part of a CIP or COP cleaning process, in which the cleaning solution or rinse water passes through the control unit, spray devices, and equipment multiple times. Typically used for the cleaning cycle of a CIP process.

Recovery

Percentage of analyte that is removed and analyzed during swab or rinse sampling procedure. Determined in lab by measuring recovery from spiked coupons.

Repeatability (ICH Q2 [107])

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Reproducibility (ICH Q2 [107])

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

Residue

Material remaining on equipment surfaces after the cleaning process.

Revalidation

Validation of a previously validated system that has been changed or modified. Revalidation can be performed as the result of a change to the system, or a time based assessment.

Riboflavin Testing

Procedure for testing coverage of the spray from a spray device. Done by coating the interior surfaces with dilute solution of riboflavin, running a CIP cycle with water, and examining surfaces with a UV light.

Rinse Sampling

Procedure for sampling involving flooding the surfaces with rinse solution to effectively remove target residues. The rinse solution is then analyzed for the target residue.

Risk (ICH Q9 [22])

Combination of the probability of occurrence of harm and the severity of that harm (ISO/IEC Guide 51).

Robustness (ICH Q2 [107])

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Ruggedness – see **Intermediate Precision**

Safety Limit (SL) – may be called **Acceptable Residue Limit (ARL)**, also known as **Cleaning Safety Limit**

Represents the acceptable cleaning limit based on HBELs corresponding to a safe amount of residue in the next product dose (i.e., DP) or batch (i.e., DS).

Shadow Area

Any area that does not receive adequate cleaning solution from the spray device because of an impediment (agitator shaft, baffle, etc.) within the process vessel.

Soil

Material on equipment surfaces to be removed by the cleaning process.

Specificity (ICH Q2 [107])

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Swab

Sampling tool that comprises a textile, fiber, or foam on the end of a moderately flexible handle. An example is knit polyester on a polypropylene handle.

Swab Sampling

Procedure for sampling surfaces involving wiping the surfaces with a swab, typically saturated with water or another sampling solvent, to remove residues from a surface. The swab is then desorbed, and a chemical analysis is performed on the desorbed material.

Total Organic Carbon (TOC)

A non-specific analytical procedure that involves oxidizing the residue to carbon dioxide, and then measuring the generated carbon dioxide.

Worst Case

Those conditions within normal parameters most likely to give failure. For processing purposes, “worst case” means those values of normal operating parameters most likely to cause process failure. For sampling locations, “worst case” means those equipment locations most likely to have higher levels of residues after cleaning. For sampling recovery, “worst case” means those procedures, within normal sampling parameters, most likely to give poorer percent recovery. For grouping strategies, “worst case” means selecting those products or acceptance limits most likely to give failing results.

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