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Points to Consider for Biotechnology Cleaning Validation



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Points to Consider for Biotechnology Cleaning Validation Task Force

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1.0 Introduction

Cleaning validation plays an important role in reducing the possibility of product contamination from biopharmaceutical manufacturing equipment. It demonstrates that the cleaning process adequately and consistently removes product residues, process residues and environmental contaminants from the cleaned equipment/system, so that this equipment/system can be safely used for the manufacture of defined subsequent products (which may be the same or a different product). As used in this Technical Report, “product” may be a drug product, bulk active, intermediate, or another type of formulation. If “drug product” is intended, that terminology will be utilized. While cleaning validation for biotechnology manufacturing has many of the same elements as for other pharmaceutical manufacturing, there are enough differences such that a separate Technical Report focusing on biotechnology cleaning validation is appropriate.

Previous PDA documents on cleaning validation, including the 1998 PDA *Technical Report No. 29, Points to Consider for Cleaning Validation* and the 1996 monograph *Cleaning and Cleaning Validation: A Biotechnology Perspective* provide valuable insights for biotechnology manufacturers. (1,2) However, this report presents more updated information that is aligned with life cycle approaches to validation and the International Conference on Harmonisation (ICH) guidelines Q8(R2), *Pharmaceutical Development*, Q9, *Quality Risk Management*, and ICH Q10, *Pharmaceutical Quality System*. (3-6) This report also aims to present information in a way that readers can easily utilize to assist in creating a cleaning validation program for their equipment and facilities.

The Biotechnology Cleaning Validation Task Force was composed of European and North American professionals from biotechnology manufacturers, cleaning chemical suppliers, regulatory agencies and consulting companies. This report also underwent a global, technical peer review to ensure concepts, terminology, and practices presented are reflective of sound science and can be used globally.

Note: For ease of use, this Technical Report includes a list of acronyms used throughout the document. Refer to **Section 16.0**.

1.1 Purpose/Scope

The focus of this Technical Report is on biotechnology manufacturing. Biotechnology manufacturing includes bacterial and cell culture fermentation. While some might exclude plasma fractionation and egg-based vaccine manufacturing from the strict definition of biotechnology, many of the practices and guidance in this report are applicable to plasma fractionation and egg-based vaccine manufacturing. Therefore, examples given will be for biotechnology manufacturing. We have also included a life cycle cleaning validation approach, including design/development of the cleaning process, process qualification (the protocols runs), and ongoing validation maintenance. These practices and the associated guidance in this Technical Report are based on technical considerations and should be applicable in all regulatory environments.

The intent of this Technical Report is not to provide a detailed plan or detailed road map for a biotechnology manufacturer to perform cleaning validation. Rather, as the title suggests, it presents “points to consider” as one designs a cleaning validation program for biotechnology manufacturing based on an understanding of one’s manufacturing and cleaning processes. In cleaning validation, there are generally *multiple* ways to accomplish the same goal of a compliant, scientifically sound and practical cleaning validation program. Where options are given, the rationales for such options are also generally given. The Biotechnology Cleaning Validation Task Force that developed this document hopes that it will be used in that spirit. Based on an understanding of the unique nature of any individual situation, different approaches or additional issues should also be considered.

This report should be considered a resource to help guide the development or evaluation of a cleaning validation program. It is not intended to establish mandatory standards for cleaning validation. It is intended to be a single-source overview for biotechnology manufacturers that complements existing guidance and reference documents, listed in **Section 13.0**. The reader should also be aware that a specific topic may be discussed in several sections of this Technical Report. Therefore, a more complete perspective may be obtained by considering all relevant sections about a certain topic.

2.0 Glossary of Terms

Acceptable Daily Intake

An amount of a substance administered or consumed on a daily basis that is considered a safe level

Analyte

A substance (usually a residue) for which an analysis is being performed

Blank

An analytical sample taken to establish the background value for an analytical measurement which may be subtracted from an experimental value to determine the “true” value

Campaign

The processing of multiple lots or batches of the same product serially in the same equipment

Changeover

The steps taken for switching multi-product equipment from the manufacture of one product to the manufacture of a different product

Clean

Having product residues, process residues and environmental contaminants removed to an acceptable level

Cleaning Process

A process that is used to remove any product, process related material and environmental contaminant introduced into equipment as part of the manufacturing stream

Cleaning Validation

The documented evidence with a high degree of assurance that a cleaning process will result in products meeting their predetermined quality attributes

Cleaning Verification

A one-time sampling and testing to ensure that specified equipment has been properly cleaned following a specific cleaning event

Coupon

A small, generally flat portion of a defined material of construction (such as stainless steel or PTFE), typically used for laboratory cleaning evaluations and/or for laboratory sampling recovery studies

Degradation

The breakdown (usually chemical) of material during manufacture (including during and after the cleaning process)

Dry Equipment

No visible water pool evident in the equipment or line when viewed under appropriate lighting conditions

Equipment Train

The sequence of equipment through which a product is produced or processed

Grouping Strategy

A strategy of establishing the similarity of cleaning processes, usually based on similar products or similar equipment, and validating the cleaning process based primarily on validation data for a representative of the group

LD₅₀

The “dose” of a material which results in 50% mortality in an animal test

Limit

A value for a residue above which a cleaning validation protocol would fail

Normal Dose

The therapeutic dose of a product as given on the approved product labeling

Recovery Study

A laboratory study combining the sampling method and analytical method to determine the quantitative recovery of a specific residue for a defined surface

3.0 Cleaning Process Design and Development

3.1 Introduction

The cleaning process requires design and development *prior to* implementation in a manufacturing plant to ensure the cleaning process and equipment are acceptable for use. Additionally, the concept of “Design Space,” recently introduced as an approach to the development of pharmaceutical processes, is discussed and applied to the development of cleaning processes.

The operational parameters that describe the cleaning process (such as cleaning agent, concentration, contact time, temperature, soil characteristics and soil condition), as well as specifics about the cleaning equipment, automated cleaning pathways, the sequence of cleaning steps, and flow rates during each step, should be determined prior to implementation.

Generally, the establishment of acceptable conditions (or confirmation of acceptable conditions for new soils being introduced to the manufacturing plant) follows a standard progression of activities – beginning with identification of control variables, cleaning measurements, and performance criteria. Laboratory (scale-down) experimentation, analogous to process characterization, and specific equipment requirements provide the necessary data to establish cleaning parameter control ranges.

This section describes the application of operational parameters and measurements, the design of laboratory scale experiments, the selection of appropriate test soils, and the scale-up for cleaning the manufacturing equipment.

3.2 Cleaning Process Controls (Inputs) and Measurements (Outputs)

3.2.1 Cleaning Cycle Design

Cleaning processes are comprised of multiple steps. Each step in the process has a function and a set of parameters that are controlled within defined ranges to ensure effective soil (and cleaning agent) removal. Steps in a typical cleaning cycle for a biotechnology product are outlined in **Table 3.2.1**. Details of the cleaning processes may vary from site to site and for different types of process equipment. Differences may include the use and type of detergents, the presence of an acid cleaning step, the concentration of cleaning agents, contact time of cleaning agents on equipment, feed pressure or flow rate, cleaning temperature, and required length or volume of rinse steps.

Table 3.2.1 Cleaning Process Steps (Examples)

STEP	FUNCTION	COMMENTS
Pre-Rinse	Removal of soluble and non-adhering residues	Reduction of soil load prior to primary cleaning step. Often performed at ambient temperature to avoid denaturation of residual proteins.
Alkaline Cleaning Solution	Removal of soluble and dried residues, solubilization of soils by degradation, heat, and/or wetting with detergents	Primary step for soil and bioburden removal. Generally performed at elevated temperatures. May include alkaline detergents or alkali hydroxides. May not be required depending on soil composition.
Water Rinse	Removal of alkaline detergent and suspended or solubilized soils	May not be as thorough as final rinse if prior to acid rinse.
Acid Rinse	Neutralization of residual alkali, additional cleaning of acid-soluble soils	May not be required depending on soil composition.
Final Water Rinse	Removal of all cleaning agent and product residues	Generally performed at elevated temperatures.

3.2.2 Physical-Chemical Aspects

There are four principal cleaning input parameters that can be varied for each step in the cleaning process. These four parameters are typically referred to as TACT (Time, Action, Concentration and Temperature). These four variables are interrelated and have a direct relationship on the success of each phase in the cleaning cycle. For example, cleaning agents may be heated to increase their effectiveness. The effect of each of these variables on soil removal should be determined, and acceptable ranges should be established as part of the cleaning development effort (soil type and condition are additional inputs that are discussed in **Section 3.5**).

Time is defined as the length of time for the cycle step. There are two typical methods for defining and measuring time during a cycle step, direct and indirect. Using the direct method, a cycle step counter that is part of the control system is used to measure the cycle step time. Time also may be measured indirectly. For example, for a rinse step, volume is sometimes tracked instead of time because the volume and flow rate define a time. For final water rinse, it is also common to add additional requirements, such as a specified conductivity level.

Action is the mechanism used to deliver the cleaning agent. This mechanism may be characterized as soak, scrubbing, impingement or turbulent flow. Agitation enhances the chemical actions of the cleaning agents and helps to increase the effectiveness of the cleaning process. Manual cleaning typically includes soaking or scrubbing as the action to achieve cleaning. Automated cycles typically employ impingement and/or turbulence as a cleaning action. The type of cleaning action should be identified for each cleaning process. The velocity (flow rate) of the cleaning and rinse fluids traveling through the equipment is an operational parameter that should be specified and verified at each step.

in the cleaning process. Spray devices have minimum and maximum flow rate requirements, and piping should be flushed at a speed sufficient to assure adequate flooding and turbulence.

Cleaning agent concentrations directly affect the success of the cleaning process. Cleaning chemicals are available in concentrate forms that are diluted and used in cleaning cycles. Effectiveness of the cleaners may be related to their concentration. Too little cleaning agent might result in failure to remove the soil from equipment, and too much detergent can result in difficulty in removing cleaning agent residues, requiring excessive rinsing. In general, the greatest effectiveness of alkaline cleaners is achieved at elevated temperatures with agitation or turbulence over extended periods of time. Although there is some risk of denaturing residual protein onto the surfaces of the equipment at high temperatures, thereby making it more difficult to clean, it is typically minimized by performing an appropriate pre-rinse at ambient temperature prior to the caustic chemical wash. Chemicals are also costly, both in their purchase and disposal, thus determining the correct concentration of cleaning agent required to ensure cleanability is important. The addition of a cleaning agent to an automated system must be designed for reproducibility. Regardless of the method of addition, confirmation of the cleaning agent concentration helps verify consistency. For automated cleaning processes, the easiest means to verify cleaning agent concentration for highly alkaline or acidic cleaning agents is by conductivity.

A process should be in place to detect anomalies in detergent concentration based on the mechanism by which chemical makeup is performed. For example, some systems control chemical addition by volume and use conductivity as a confirmation. An alarm would be triggered if the conductivity is outside a preset range. The allowable range should be supported by cleaning development data.

Selection of the cleaning agent should consider various aspects, including soil type, ease of removal, and need for chelating agents.

The optimal temperature ranges will vary for the different steps of the cleaning process. Initial water rinses are typically performed at ambient temperatures to minimize denaturing effects on proteins and maximize the dilution effects. Cleaning agents are typically heated to increase their effectiveness. Final rinse water steps may be performed at high temperatures to increase both the drying rate and the solubility of any process or cleaning agent residues.

3.3 Measurements Used to Determine Cleaning Effectiveness

Cleaning effectiveness may be determined by the inspection and analytical methods described in **Section 6.0**. They include visual inspection, analytical techniques for measuring removal of manufactured product, cleaning agent, bioburden and endotoxin. Depending on the purpose and the design/ development phase, these may be on-line measurements and/ or may be off-line measurements of rinse or swab samples.

3.4 Equipment and Plant Design Considerations

3.4.1 Piping

Piping of the equipment being cleaned and of the CIP skid should be sloped continuously to ensure maximum drainability of the lines. If supply and/ or return loop headers are used, the loop must be designed such that liquid flows in both parts of the loop at adequate speeds. If this is not achieved, one part of the loop may become a functional deadleg.

The pressure drop in the piping also needs to be considered. The CIP skids are often located remotely from the process area, and the length of the distribution piping results in a total pressure drop that can be significant. The greatest challenge is sizing the distribution piping when the supply flow rate set points in the system vary by more than twofold.

3.4.2 Automated vs. Manual Systems

Use of automation provides consistent and robust control and monitoring of CIP cycles and parameters (such as time, flow rate or pressure, cleaning agent concentration, and temperature). Manual systems require more detailed operating instructions and increased operator attention during use.

3.4.3 Centralized CIP vs. Discrete Cleaning of Isolated Equipment

Centralized CIP systems can provide a single location for handling cleaning agents and can reduce the plant requirements for cleaning-related equipment (pumps and tanks) and instrumentation. However, centralized systems often require more complex piping designs and may complicate desires to segregate parts of the process (e.g., upstream and downstream operations or pre- and post-virus removal steps in mammalian cell processes). Some process equipment (e.g., reusable membrane systems and chromatography columns) may require special cleaning agents that are different than those used for the rest of the process equipment. For these systems, discrete CIP or CIP systems (including portable CIP systems) that are integrated into the process skids may be desirable.

The design of centralized CIP systems should consider the potential for carryover of product residues between process steps, between products being manufactured concurrently in multi-product facilities, and between different products after a product changeover. To address the potential for product carryover, central CIP systems are often dedicated to one part of the manufacturing plant (e.g., upstream through product clarification steps or one process train in a multi-train plant).

3.4.4 Clean Out of Place (COP)

Small parts, containers, and other portable process equipment that are difficult to clean in place are often disassembled and cleaned in COP stations, washers or baths. Where COP is used, care must be taken in handling the parts after cleaning and in identifying parts for correct reassembly. COP may be performed in automated washers or baths or by manual cleaning.

3.5 Soil Evaluation and Categorization

3.5.1 Soil Categories

There are a large variety of substances that contact process equipment surfaces during the manufacture of biopharmaceutical products. They include: fermentation and cell culture media; cells and cellular products, such as proteins and nucleic acids; organic and inorganic acids, bases and salts; process additives, such as antibiotics, surfactants, glycols, polyamines, sugars, plant or animal hydrolysates; and cleaning agents such as detergents, acids and bases.

Cleaning processes and cleaning validation must be designed and tested to address this wide variety of potential process soils. These tasks may be simplified by creating categories of soils and selecting representative soils for testing and tracking during the development and validation of cleaning processes. An example of process soil categories is shown in **Table 3.5.1**.

Table 3.5.1 Process Soil Categorization (Example)

Category	Representative Soil	Applicable Process Equipment
Fermentation / Cell Culture Soils	Unconditioned (unused) fermentation/culture media	Media preparation equipment
		Media storage and feed tanks
	Conditioned (post-fermentation, cell-containing) culture media	Inoculum preparation equipment
		Seed reactors (fermentors)
		Production reactors (fermentors)
		Cell processes and clarification equipment
	Conditioned (post-fermentation, cell-free) culture media	Post-clarification product processing and storage equipment
		Product capture equipment
Downstream and Formulation/Fill Soils	Aqueous salt solution containing product	Purification equipment
		Concentration and buffer exchange equipment
	Solution containing product and formulation components (e.g., excipients and surfactants)	Formulation equipment
Buffer Preparation Soils	Aqueous salt solution without product	Final sterile filtration equipment; product filling equipment
		Buffer preparation equipment
Cleaning Agent Soils	Cleaning agent, including surfactant and alkali/acid	Buffer filtration equipment and hold tanks
		Process equipment

The final selection of a representative soil within a process stream should be based on the similarity of the physiochemical properties of the soils. In many circumstances, categories may be combined, and the number of representative soils used for development activities may be further reduced.

3.5.2 Soil Removal

Soils may be removed by physical and/or chemical means. Physical removal may be accomplished by the diffusion of soils away from the surface (static soaking) or by convection, whereby energy from cleaning solution flow is used to transport soils into the fluid stream. Physical removal is dependent on soil size and its degree of adhesion to the equipment surface.

Chemical cleaning mechanisms include solubility, emulsification, wetting, chelation, dispersion, hydrolysis and oxidation. Cleaning agents are generally chosen for their ability to remove process soils by one or more of these mechanisms. In some cases, multiple cleaning steps may be used in order to take advantage of different chemical cleaning mechanisms. For instance, alkaline detergent for solubilization and emulsification may be followed by a sodium hypochlorite solution for the oxidation of protein soils. It should always be kept in mind that the more aggressive the cleaning solvents are (e.g., sodium hypochlorite solution), the more corrosion might occur. The right choice of materials for cleaning purposes is part of the engineering phase.

Factors affecting “cleanability” also include the surface type, the surface finish, the surface geometry,

the soil type and the soil level. Process surfaces typically encountered in biopharmaceutical manufacturing equipment are listed in **Table 3.5.2**.

Table 3.5.2 Surface Materials for Biopharmaceutical Production Processes

Material	Description
Stainless steel, Hastelloy® [†] , other alloys	Reactors, tanks, piping, other large-scale equipment
Glass, ceramic	Flasks, beakers, cylinders
Polyethylene	Carboys
Polypropylene	Carboys
Fluorocarbons (PTFE; FEP)	Gaskets, hoses, piping, small containers
PETG Plastic	Culture vessels
Polycarbonate	Small containers
Silicone	Tubing, gaskets, O-rings
Fluoroelastomer	Gaskets, O-rings
EPDM	Gaskets, O-rings

[†] Hastelloy is a registered trademark of Haynes International, Inc.

Surface finish also affects the removal of soils. Rough surfaces provide more area for soil contact and may contain cracks and crevices that are difficult for the cleaning agent to penetrate. Therefore, the interior surfaces of stainless steel process equipment are typically treated (e.g., electropolishing) to smooth and polish rough surfaces.

The ease with which a soil is released from the equipment surface by one of the mechanisms described above determines its cleanability. Soil response to a particular cleaning mechanism may influence the choice of cleaning agent and cleaning conditions. Attachment to surfaces can be by a combination of van der Waals forces, electrostatic effects and other forces. The time that the soil resides on the equipment can also influence the difficulty of soil removal. Fresh soils are generally easier to remove than soils that have been allowed to dry on the surface. The planned time between soiling and cleaning must be considered when designing the cleaning studies to simulate the dirty hold time with coupons.

High soil levels can complicate removal by saturating the cleaning solvent or by depleting surfactants or other components of the cleaner (e.g., oxidizers or emulsifiers). This may limit the minimum cleaning volumes and should be considered in cleaning cycle design when high soil levels are anticipated.

3.5.3 Cleaning Comparability Based on Soil and Surface

Laboratory testing often includes screening a matrix of soils and relevant process surfaces. Screening experiments are designed to test soil removal capability using representative soils (**Table 3.5.1**) and coupons of relevant surface materials (**Table 3.5.2**). Cleaning conditions can be selected based on the results for the soil-surface combination encountered in the production equipment.

3.5.4 Soil Selection for Laboratory Evaluations

Care should be taken in the choice of soils and soil conditions used for the selection of cleaning agents during laboratory evaluation. The soils should be representative of the soils on equipment in the manufacturing plant, including the chemical and physical (dried, baked) nature of the soils.

Solutions or suspensions of soils selected for experimentation are generally coated on coupons representing the process contact surfaces and are dried to simulate the soil condition on the process equipment prior to testing for removal with cleaning agents. The representative soils generally should include conditioned culture media and the product solution. Other relevant soils may also be included, as mentioned above and outlined in **Table 3.5.1**. The number of representative soils will vary with an organization's experience and history as well knowledge about the content and cleanability of the various process steps.

3.6 Performing Cleaning Development Experiments

Biotechnology processes generally involve product contact with a variety of materials (**Table 3.5.2**). Laboratory evaluation of the interaction between product and surfaces can be performed using test coupons made of the surface of interest under simulated cleaning conditions. Based on the process details, appropriate materials of construction with the appropriate surface finish characteristics should be selected for use in lab-scale cleaning experiments. To minimize the number of experiments, it may be sufficient to include only those surfaces that are expected to be the most difficult to clean (based on prior knowledge and risk assessment tools). Stainless steel coupons are the most common choice, as they represent a majority of equipment surfaces in a production facility. Non-electropolished stainless steel coupons with a representative or worse surface finish compared to vessel surfaces may be preferred for lab evaluations.

Preparation of coupons typically involves the use of a cleaning regimen in order to ensure that all coupons are uniformly cleaned at the start of the experiment. This also helps to ensure that any foreign material deposited on the coupon surface during the fabrication process is removed to minimize any interference with the process soils or cleaning agent. The coupons are then completely dried before spotting them with soils, which may be the cell culture/fermentation fluid, harvested cell culture fluid, bulk drug active, and/or the final drug product formulation. It is important that the spotting of liquid onto each coupon be kept consistent to minimize experimental variability. The coupons are then dried for a fixed time to simulate the soiled equipment surfaces at the time of cleaning before they are subjected to the lab-scale cleaning process. That fixed time is generally the desired dirty hold time or a longer time.

The purpose of the experiment could be to make one or more determinations related to cleanability: comparison of the various materials of construction for a given soil, comparison of different process streams for a given surface, comparison of different cleaning conditions (such as concentration of cleaning agent and temperature), comparison of different products for the same process step and surface, or a combination of these. The outcome of these studies can be analyzed to create the "design space" for cleaning. In any case, it is important that the performance of the cleaning process in the lab represents the performance in the pilot plant or larger scale process. Key operational parameters, such as temperature, time, mode of action and concentration, are controlled to mimic what is used in the manufacturing plant. If it is difficult to simulate the actual process conditions in the lab, conditions representing a worst-case scenario should be employed. The laboratory studies can also be used to challenge the cleaning process by modifying different variables of the cleaning process to further outline the design space.

Evaluation of performance for cleaning design space studies can utilize the various analytical methods listed in **Section 6.0**.

3.6.1 Parameter Selection

A variety of parameters can impact the performance of a cleaning regimen. These include the nature and strength of the interactions between the product and the surface; the nature of the interaction between the cleaning agent and the soil; time (dirty hold time, time for each cleaning cycle); cleaning agent and concentration; temperature; cleaning action (flow properties, e.g., stagnant, laminar or turbulent, and pressure; and properties of the liquid (ionic strength, pH, components, viscosity, density, etc.). All of these except the cleaning action are independent of the equipment. The selection of parameters to be examined in an experimental study should be done on a case-by-case basis. The larger the number of parameters that need to be evaluated, the more the number of experiments that are required to understand the impact of the parameters and their interactions. On the other hand, if too few parameters are picked, the resulting conclusions in terms of identifying the important operational parameters and their ranges are likely to be erroneous, since important effects might be ignored.

Use of a risk analysis tool, such as Failure Mode and Effects Analysis (FMEA), may assist in prioritizing the various operational parameters for further examination (See **Section 10.0** on Risk Management). Single parameter studies that vary one parameter at a time can be designed to identify the parameters that have significant impact on the performance. One such study, conducted at bench scale, reported the concentration and temperature of the cleaning solution to be the parameters with predominant effects. (7) As discussed in the following section, single parameter studies can then be followed by Design of Experiments (DOE) to investigate the interactions between these parameters. Alternatively, if only a few parameters need to be examined, just performing a DOE to measure both the main effects and the interactions may be more resource and time efficient.

3.6.2 Parameter Interactions

The use of DOE-style experiments helps to determine the effect of varying individual parameters on cleanability, as well as provides an indication of their interaction. Statistical tools including regression analysis, leverage plots, response surface analysis and interaction profiles can be used to study both main and interaction effects. Relationships and interactions between such parameters as the temperature of the cleaning solution and the concentration of the cleaning agent may be determined. Such DOE analyses can be used to construct a multi-parameter design space for the cleaning process and to establish the ranges of operational parameters that provide acceptable cleaning process performance.

Using existing knowledge and a risk-based approach, cleaning experiments can be reduced or eliminated, for example, for transfer of a manufacturing process from one facility to another.

3.7 Cleaning Process Scale-Up

Following the selection of cleaning agents and cleaning conditions (such as temperature, contact time, cleaning agent concentration and flow stream hydrodynamics) from historical plant data (if available) and laboratory development work, the cleaning process can be implemented for use on larger scale manufacturing equipment. Determination of soil and cleaning agent residue removal is generally performed prior to formal cleaning validation. Adjustments to cleaning conditions may be made during the scale-up process based on plant experience and laboratory development studies.

3.7.1 Setting Process Controls

It is both prudent and consistent with current CGMP to establish control ranges for the cleaning process operational and performance parameters. Operational parameters for CIP include:

- Dirty hold time for equipment (time between use and initiation of cleaning)
- Flow rate and/or delivery pressure of the cleaning stream (proof of flow for any parallel flow paths)
- Cleaning agent concentration
- Duration of each step in the cleaning process (by time or volume)
- Temperature of cleaning agents and rinses
- Air flow verification during any water removal or drying steps

Instrumentation for each of these parameters should be included in the system design. Alert and action levels can be set for each parameter in order to maintain proper operation. Alert levels may be set based on expected variability of the equipment and instrumentation in the CIP system. Action levels should be set at values that permit adjustment to the equipment to avoid jeopardizing acceptable operation. Both alert and action levels should be within the acceptable ranges for each parameter. It is also reasonable to establish check times, such that an alarm notification will occur if parameters do not reach their set points (e.g., volume flow, conductivity) within the specified time.

Performance parameters should also be evaluated during scale-up. Performance parameters may include:

- Final rinse water conductivity
- Final rinse water TOC
- Final rinse water bioburden
- Final rinse water endotoxin

3.7.2 Introduction of New Soils to a Validated Cleaning System

Once cleaning processes are successfully operating in the manufacturing plant, they are monitored to ensure soil removal remains effective. When new products or significantly different raw materials are introduced to the plant, a system must be in place to ensure that the cleaning process will remain effective.

Generally, the cleaning effectiveness of the existing system for new soils can be tested by performing laboratory experiments using coupons of relevant materials (see **Section 3.6** on Lab Development). These experiments can be designed to test both the effectiveness of the proposed cleaning regimen and the relative difficulty of cleaning the new soils compared to soils that have already been introduced to the plant. If the new soils are easier to clean than the most difficult soil already being cleaned, introduction of the new material using existing cleaning procedures can be made with confidence. If the material is more difficult to clean than any of the present soils, some modifications to the current cleaning process may be required, and cleaning validation is an expectation. However, if the new soil is easier to clean, then the number of confirmatory runs needed (if any) is determined based on a risk assessment.

Each organization should have its own system for maintaining effective cleaning after the introduction of new products or raw materials.

3.8 Applying the “Design Space” Concept to Cleaning Processes

“Design Space” is the multi-dimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality. The design space concept has been introduced by the ICH (4) to describe an approach to the development and control of pharmaceutical manufacturing processes. An analogous approach can be applied to cleaning processes.

The cleaning design space for a manufacturing facility is defined through a risk- and science-based approach relying on cleaning process knowledge, product/equipment knowledge, regulations and quality practices (requirements). Similar to manufacturing process development, control and validation, cleaning process operational parameters (inputs) can be controlled to ensure predictable and acceptable performance as evidenced by appropriate measurements (outputs). The cleaning design space is represented by the range of each of the operational parameters that results in acceptable performance of the cleaning process.

Steps in defining the design space for a cleaning process may be slightly different from steps taken to define design space for a manufacturing process, in that the design space for a manufacturing process is unique to that process (a fermentation, for example). However, many biotechnology manufacturers may want to design one cleaning process for a specific equipment train that is used, regardless of the manufactured product. This may be accomplished by identifying the “worst-case” soils and defining the design space around cleaning process performance using these soils.

Specifications are developed to support the design, installation and operation of the cleaning system. Risks are identified and assessed for impacts to safety and cleaning effectiveness (e.g., severity, probability of occurrence and detectability). Parameters may be categorized based on their level of criticality, with the most critical parameters monitored closely so that the cleaning operation can be repeated if parameters are not kept within their predetermined ranges. The criticality of cleaning process operational parameters is based on laboratory studies that document the influence of each parameter on cleaning effectiveness.

Cleaning effectiveness is influenced by the following factors:

- soil type or family
- dirty hold time
- equipment and contact surface type and finish
- cleaning technology and functional specifications for the cleaning process

This information is used to drive the design requirements for the cleaning method. Cleaning validation supports the worst case range of testing. Field conditions such as the lowest flow rate, least concentration of cleaning agent, minimal contact time, minimal process temperature, and longest dirty hold time are conditions that are considered when developing an effective cleaning process. The assumption is that any cleaning process that is performed within the space defined by these conditions will be effective, reliable and consistent.

A soil evaluation (characterization) study is performed prior to the introduction of any new process into manufacturing. These studies support the design space for the range of soils that will be cleaned from the process equipment. The characterization study is conducted with material that is representative of the process or a soil that represents the worst-case condition. For example, it is typical to use harvest material for this purpose, as it is the most complex and concentrated form of

bioprocess soil. The soil type selected for a characterization study is dependent upon the impact to the manufacturing process. If a soil does not react as expected following exposure to a cleaning agent, alternate cleaning solutions should be evaluated.

Cleaning operational parameters (inputs) should be monitored to assure compliance with the established design space. Operational parameters may be set within the design space. Setting operational parameters with tighter ranges than allowed by the design space provides some flexibility in addressing deviations of operational parameters outside the control ranges.

4.0 Acceptance Limits

Cleaning validation is performed to demonstrate the effectiveness and consistency of a cleaning procedure. The rationale for selecting limits for product residues, cleaning agents and microbial contamination, as well as any other process components, should be logically based on the materials that impact the manufacturing process and the safety and purity of the product. The acceptable limits for cleaning manufacturing systems and components should be “practical, achievable and verifiable.” (8)

Limits for cleaning validation generally contain some measure related to the active protein (or other major component of interest), some measure related to the cleaning agent, some measure related to bioburden levels, some measure related to endotoxin levels, and a requirement that the equipment be visually clean. In addition, if there are any specific toxicity concerns related to the active protein or other process components (for example, cytotoxicity, allergenicity, or reproductive hazards), the manufacturer’s toxicology or pharmacology groups may determine if a modification of limits is required, or whether the use of dedicated equipment is needed.

In the discussion that follows, issues for limits are considered based on the nature of the residue and on the stage of manufacturing (e.g., bulk active vs. formulation/fill). Manufacturing stages include bulk active manufacturing (all steps resulting in the bulk active drug substance) and formulation/fill (formulation of the bulk active into a finished drug product and primary packaging of that drug product). Bulk manufacturing is further divided into upstream process steps (all process steps through harvesting) and downstream process steps (purification and following steps).

4.1 Key Issues in Limits for Actives

Biotechnology cleaning processes often involve a change of the active molecule itself, which is commonly a protein. Proteins typically are degraded to some extent by the cleaning processes commonly used in biotechnology manufacturing. The most important mechanism for degradation is summarized below.

In alkaline solutions, such as hot, aqueous solutions containing sodium or potassium hydroxide, proteins may hydrolyze to soluble oligomers or free amino acids. Ester groups on actives may be hydrolyzed to an alcohol and a fatty acid. A common example of this is saponification of fats and oils to glycerol and fatty acid anions.

Sodium hypochlorite is sometimes used in biotechnology cleaning. As a cleaning agent, it is particularly effective in removing denatured protein residues from surfaces. It is a reactive oxidizer which will degrade proteins in a more random manner to smaller fragments. A general concern with sodium hypochlorite use is its possible deleterious effect on stainless steel components. Therefore, it is critical that the rinse cycle following the use of sodium hypochlorite is adequate enough to remove any residual chloride ion before adding the subsequent acid wash.

Proteins will hydrolyze at a high pH. The parameters of time and temperature have a significant influence on protein hydrolysis. Therefore, the higher the temperature and pH, the more extensive protein hydrolysis will occur. Because the protein is typically degraded into smaller fragments and those fragments tend to be more polar, they are likely to be more water soluble and more readily removed from equipment surfaces during the washing and rinsing processes. A second effect after protein exposure to high pH solutions is a possible irreversible, significant decrease of biological activity due to hydrolysis.

Degradation of the active can be demonstrated in a laboratory study by exposing the active to the cleaning solution under simulated cleaning conditions (or less stringent conditions) and performing

analytical and/or biochemical tests on the resultant mixture.

For these reasons, in most cases biotechnology manufacturers do not directly set limits for *and* directly measure the active in cleaning validation. Because of the degradation of the active, no active protein should remain after completion of the cleaning process. It is for that reason that analytical methods like TOC (see **Section 6.0**) are used for the detection of protein residues (or their fragments). If a nonspecific method like TOC is used for the correlation to residues of the active, it should be noted that the “real” value of protein residues after cleaning may be significantly lower, as TOC measures all sources of organic carbon (and not just residues from the active protein).

4.1.1 Establishing Limits for Actives in Formulation and Final Fill

In biotechnology formulation/fill manufacturing, limits for protein actives are typically set using a carryover calculation (often called MAC, or Maximum Allowable Carryover) in the same way as for small molecule cleaning validation. Though the product is degraded (as discussed above), the calculations are based on active product. This is assumed to represent a worst-case approach if the cleaning method used in formulation/fill results in degradation of the protein active to fragments. Such calculations may be revised based on degradation considerations.

This method only applies when the therapeutic daily dose is known. For products dosed chronically, a typical calculation allows no more than 1/1000 of the minimum daily therapeutic dose of an active in the maximum daily dose of the subsequent manufactured product; the factor of 1/1000 may be modified depending on the specifics of the situation. In addition, if that calculation allows more than 10 ppm of the active protein in the subsequent drug product, a limit of 10 ppm active protein in the next drug product may be utilized. Similar criteria are included as examples in the both the U.S. FDA (8) and PIC/S guidance documents. (9)

Limits per surface area can then be calculated based on the minimum batch size of the next drug product and the shared surface area. Limits in swab and/or rinse samples can then be calculated using the sampling parameters.

When this method is used for setting limits, the limit for the active is calculated. It can then be converted to appropriate units for the analytical procedure to be utilized. For example, if the analytical procedure is TOC, the limit calculated for the active is converted to TOC based on the TOC content (percentage) of the active.

An example carryover calculation for formulation/fill is given as **Example 1** in **Section 15.0** of the Appendix.

It should be noted that limits based on carryover calculations are one example of a “science-based” method of setting limits. Some companies choose to set limits based on more stringent criteria, such as the WFI TOC specification of 500 ppb TOC. Such an approach is acceptable, but should only be used if it can be demonstrated that the WFI TOC specification is more stringent than the TOC result, as determined by a carryover calculation.

4.1.2 Establishing Limits for Actives in Bulk Manufacture

Carryover calculations used for formulation/fill are typically *not* applicable for bulk manufacture. The primary reason is that if the carryover calculation is based on the entire equipment manufacturing train surface area, limits are extremely low and are not measurable by available analytical techniques, unless the active is not degraded during the cleaning process. As previously discussed, the active

protein is, in almost all cases, degraded by the cleaning process. Therefore, even though typical specific assays for the active can measure the active at very low levels, those assays are not useable when the active is degraded. As noted above, the residues of the active are generally measured “indirectly” by measuring a property like TOC (for purposes of this report, TOC will be used as an example of such analytical methods, although assays like Total Protein could also be used). Typical quantitation limits for TOC for cleaning validation purposes are on the order of 100-500 ppb carbon, which is equivalent to about 200-1,000 ppb protein for proteins containing about 50% carbon. Therefore TOC cannot be used as an analytical method if limits are based on a carryover calculation using the surface area of the *entire* equipment train.

Some companies choose to use a carryover calculation for a limited surface area, such as for the last manufacturing vessel or for all of the manufacturing equipment after the last purification process. Such a modification may result in a carryover limit, which possibly could be measured by TOC. One rationale for such an approach can be found in ICH Q7, *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients*; Section 12.7 of the guide says that early processing steps do not require validation if subsequent processing steps remove those residues. (10) The various processing steps after fermentation and processing, including chromatographic purification and ultrafiltration, can be expected to remove those residues of the active from earlier cleaning steps, since those residues are typically smaller fragments. This generally has not been demonstrated for a specific active but is a reasonable supposition based on an understanding of degradation and the various purification processes utilized in a given manufacturing process. Inactivation of active protein can be considered in carryover calculations with rationales. This results in a conservative limit, because it is assumed that the degraded fragments have significantly less pharmacologic effect and/or safety concern compared to the native protein.

Because of these measurement issues, and because of the degradation issues, a more common technique for setting limits for residues of the active is based on process capability. This is not a true process capability study, but is based on what has been and can be achieved by conventional cleaning procedures in biotechnology manufacturing. These limits are typically based on the TOC values of any sample, whether rinse or swab. As a general rule, the limits may be more stringent following the purification process. TOC limits upstream are typically less stringent, because the product cleaned from the equipment surfaces has more extraneous materials (such as cellular materials). Typical values established as acceptance criteria by manufacturers are 1-2 ppm TOC for downstream processes and 5-10 ppm TOC for upstream processes. As in other cleaning validation protocols, it should be noted that typical values *achieved* are significantly below established limits.

Exceptions to this practice are for product-dedicated materials like ultrafilter membranes and chromatography resins. These are part of the downstream processing, but limits may be set higher. This higher level is acceptable when these items are dedicated to the manufacture of *only one product*.

An example carryover calculation for bulk active manufacture (assumes the entire manufacturing train is the shared surface area) is given as **Example 2** in **Section 15.0** of the Appendix. The purpose of that illustration is to demonstrate the low (and unmeasurable) TOC values that are achieved in such calculations.

4.1.3 Limits Based on Toxicity Data

An alternative to establishing limits for the active for either formulation/fill (**Section 4.1.1**) or bulk manufacture (**4.1.2**) is to establish limits based on toxicity calculations. These toxicity calculations for the active are similar to toxicity calculations for cleaning agents covered in **Section 4.2.1** Toxicity

calculations typically involve the use of the short-term toxicity data for the active, ultimately to arrive at an ADI (Acceptable Daily Intake). Calculations should preferably be based on toxicity data by the same route of administration (such as intravenous studies if the drug product is given by injection). If the data are based on oral toxicity, additional safety factors may be required for the cleaning of injectables. As a general observation, such calculations for actives based on safety considerations generally result in higher limits as compared to calculations based on dosing. Furthermore, if the active protein is degraded, the more relevant toxicity data is not the toxicity of the native protein, but the toxicity of the *degraded fragments*, which is often assumed to be less of a safety concern (although toxicity studies on degraded fragments are typically not performed).

4.2 Limits for Cleaning Agents

Limits for cleaning agents will also depend on the stage of manufacturing (formulation/fill vs. bulk active manufacture). Typical cleaning processes for biotechnology involve either a caustic wash followed by a phosphoric acid step, or an alkaline detergent followed by an acidic detergent. Each of these situations will be handled separately.

4.2.1 Limits for Commodity Chemicals

If only commodity chemicals such as sodium hydroxide and phosphoric acid are used for cleaning, it is common practice to set limits for these indirectly as a conductivity value. Preferably, an acceptable level of sodium hydroxide or phosphoric acid is established based on toxicity carryover calculations or based on the effects on process parameters. The conductivity limit is then set at a level equivalent to that concentration at a specified temperature. It is typically the case that the conductivity limit established in this way is well above the conductivity limit for WFI at the same temperature. Therefore, limits are based on either WFI specifications or on a slightly higher value (such as 5 $\mu\text{S}/\text{cm}$). The rationale for this is that it is more stringent than the “scientific” calculation allows. Furthermore, in many cases, phosphate ions and/or sodium ions may be part of any subsequently manufactured product. Therefore, carryover of small amounts is not significant. It should also be noted that calculations based on toxicity of the commodity chemicals are extreme, since sodium hydroxide is not carried over into a final product as sodium hydroxide, and phosphoric acid is not carried over into final product as phosphoric acid. If such chemicals were carried over intact at high concentrations, process checks (such as a significant change in pH) would also cause a non-conformance. Thus, the purpose is not to confirm compliance with WFI specifications, but rather to confirm low amounts of a cleaning agent. The rationale for allowing a conductivity limit slightly higher than the WFI limit is that the WFI limit applies to water in the recirculating WFI loop. As soon as the water is taken out of that loop and passed through clean equipment (particularly through spray devices where it can pick up carbon dioxide from the air), there is no expectation that it will *necessarily* meet the WFI conductivity limit.

4.2.2 Limits for Formulated Cleaning Agents

For *formulation/fill* manufacturing, limits for residues of formulated cleaning agents (which may contain a variety of organic components in addition to inorganic hydroxide) are typically set based on a carryover calculation using the short-term toxicity (LD_{50}) data for that formulated cleaner. Such toxicity information may be supplied by the cleaning agent manufacturer or may be calculated using worst-case assumptions based on an analysis of the formulation components. A typical calculation is given in **Example 3** of **Section 15.0** of the Appendix. For the determination of the limit in the next drug product, a default value for the formulated cleaning agent may also be used if it is more stringent