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Points to Consider for Biotechnology Cleaning Validation

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对于生物制品清洁验证观点思考

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Points to Consider for Biotechnology Cleaning Validation Task Force

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The content and views expressed in this Technical Report represent the work of the Task Force volunteers and are not necessarily the views of the organizations they represent.

Points to Consider for Biotechnology Cleaning Validation

生物制品清洁验证观点

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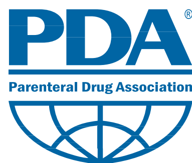

Table of Contents

1.0 INTRODUCTION 介绍 **3**

1.1 Purpose/Scope 目的/范围 3

2.0 GLOSSARY OF TERMS 术语词汇 **5**

3.0 CLEANING PROCESS DESIGN AND DEVELOPMENT 清洁工艺设计和开发 **6**

3.1 Introduction 介绍 6

3.2 Cleaning Process Controls (Inputs) and Measurements (Outputs) 清洁工艺控制（输入）和测量（输出） 6

3.2.1 Cleaning Cycle Design 清洁程序设计 6

3.2.2 Physical-Chemical Aspects 物理-化学 方面 7

3.3 Measurements Used to Determine Cleaning Effectiveness 测量用于决定清洁效率 8

3.4 Equipment and Plant Design Considerations 设备和厂房设计考虑 8

3.4.1 Piping 管道 8

3.4.2 Automated vs. Manual Systems 自动VS手动系统 9

3.4.3 Centralized CIP vs. Discrete Cleaning of Isolated Equipment 集成CIP vs. 独立设备的离散清洁 9

3.4.4 Clean Out of Place (COP) 离线清洁 9

3.5 Soil Evaluation and Categorization 污物的分类和评估 9

3.5.1 Soil Categories 污物分离 9

3.5.2 Soil Removal 污物去除 10

3.5.3 Cleaning Comparability Based on Soil and Surface 基于污物和表面的清洁比较 11

3.5.4 Soil Selection for Laboratory Evaluations 实验室评估对油选择 12

3.6 Performing Cleaning Development Experiments 清洁性能开发试验 12

3.6.1 Parameter Selection 参数选择 13

3.6.2 Parameter Interactions 相互使用参数 13

3.7 Cleaning Process Scale-Up 清洁工艺放大 13

3.7.1 Setting Process Controls 设置工艺控制 14

3.7.2 Introduction of New Soils to a Validated Cleaning System 验证的清洁系统的新污染物介绍 14

3.8 Applying the "Design Space" Concept to Cleaning Processes 应用于“ 设计空间 ”概念的清洁程序 15

4. 0 ACCEPTANCE LIMITS 接受限度 **17**

4.1 Key Issues in Limits for Actives 活性物质的关键温度-限度 17

4.1.1 Establishing Limits for Actives in Formulation and Final Fill 对配方和成品的活性活性物质建立限度 18

4.1.2 Establishing Limits for Actives in Bulk Manufacture 对来自原料工序的活性物质建立限度 18

4.1.3 Limits Based on Toxicity Data 19

4.2 Limits for Cleaning Agents 清洁代表物的限度 20

4.2.1 Limits for Commodity Chemicals 化学物质限度 20

4.2.2 Limits for Formulated Cleaning Agents 配方限度 清洁代表物 20

4.3 Bioburden Limits 生物限度 21

4.4 Endotoxin Limits 内毒素限度 21

4.5 Visual Clean Criterion 目视清洁标准 21

4.6 Modifying Limits 限度更改 22

5.0 SAMPLING METHODS 取样方法 **23**

5.1 Sampling Method Selection 取样方法选择 23

5.1.1 Direct Sampling Methods 直接的取样方法 23

5.1.2 Rinse Sampling 淋洗取样 23

5.1.3 Swab Sampling 棉签擦拭取样 24

5.1.4 Comparison of Swab and Rinse Sampling 棉签擦拭和淋洗水取样比较 25

5.2 Placebo Sampling 安慰剂取样 26

5.3 Sampling for Microbial and Endotoxin Analysis 微生物和内毒素分析取样 26

5.4 Sampling Recovery Studies 回收率研究取样 26

5.4.1 General Considerations 综合考虑 26

5.4.2 Swab Recovery 棉签回收 27

5.4.3 Rinse Recovery 淋洗水回收 27

5.4.4 "Recovery" in Visual Inspection 目视检查“ 回收率 ” 28

5.4.5 Recovery for Bioburden and Endotoxin Sampling 生物指示剂和内毒取样回收率 28

5.5 Training and Qualification of Samplers 取样人培训和确认 29

5.5.1 Key Issues for Training for Swab Sampling 棉签取样关键问题培训 29

5.5.2 Key Issues for Training for Rinse Sampling 淋洗取样关键问题培训 30

5.5.3 Training for Visual Inspection 目视检查培训 30

6.0 ANALYTICAL METHODS 分析方法 **31**

6.1 Specific Analytical Methods 定性分析方法 31

6.2 Impact of Inactivation/Degradation of the Active 活性物质失活/降解的影响 31

6.3 Nonspecific Analytical Methods 非定性分析方法 32

6.3.1 Total Organic Carbon (TOC) TOC 32

6.3.2 Total Protein 总蛋白 33

6.3.3 Conductivity 电导率 33

6.3.4 Visual Inspection 目视检查 34

6.4 Microbial Test Methods 微生物测试方法 35

6.4.1 Endotoxin 内毒素 35

6.4.2 Bioburden 微生物 35

6.5 Analytical Method Validation 分析方法验证 35

6.5.1 General Principles 原则 36

6.5.2 Compendia Methods 方法总结 37

6.5.3 Visual Inspection 目视检查 37

6.5.4 Bioburden Methods 微生物方法 37

6.5.5 Use of a Contract Laboratory 合同实验室的使用 37

7.0 CLEANING VALIDATION PROTOCOLS 清洁验证方案 **39**

7.1 Cleaning Verification Protocols 清洁确认方案 39

法规变更的关键问题
7.2 Key Issues Based on Regulatory Changes 39
7.2.1 Number of Runs in a Protocol ... 方案中的测试次数 39
7.2.2 Worst-Case Process Conditions........ 39
工艺条件的最差问题

8.0 MAINTENANCE OF VALIDATED STATE 验证的维护 ...41
8.1 Critical Parameter Control 关键参数控制...... 41
8.2 Control by Cycle Feedback 通过周期反馈控制 41
8.3 Process Alarms 工艺报警 41
8.4 Change Control 变更控制 42
8.5 Evaluation of Cumulative Changes 累计变更评价 42
8.6 Periodic Monitoring 定期监控 43
8.7 Trending 趋势 43

9.0 MASTER PLANNING FOR CLEANING VALIDATION 清洁验证主计划 44
9.1 Elements of a Comprehensive Plan 综合计划表 44
9.2 Harmonization of Site Cleaning Programs 清洗程序规划 45
9.3 Cleaning Validation Activities as a Function of Clinical Stage 清洁验证活动是一个临床阶段功能 45

10.0 RISK ASSESSMENT AND MANAGEMENT 风险评估和维护 47
10.1 Introduction 介绍 47
10.2 Techniques and Tools for Risk Management and Assessment................. 48
风险管理和评估的技术和工具

11.0 SPECIAL CONSIDERATIONS 特别注意事项 49
11.1 Grouping/Family Approach 分组方法......... 49
11.1.1 Product Grouping 产品分组......... 49
11.1.2 Equipment Grouping 设备分组 49
11.1.3 Introduction of a New Product or New Equipment Into a Group... 49
加入组的新产品或设备介绍
11.1.4 Conclusion 结论 50
11.2 Cleaning Agent Issues 清洁剂问题........... 50
11.2.1 Sodium Hydroxide Wash 氯化钠清洗 50
11.2.2 Acid Wash 酸洗........................ 50
11.2.3 Formulated Detergents 配制清洁剂 50
11.2.4 Issues in Selection 选择问题........ 51
11.3 Special Equipment Issues 专用设备问题 51
11.3.1 Chromatography Columns 色谱柱 51
11.3.2 Tangential Flow Filtration (TFF) Filter Systems 正切流流动过滤 过滤系统.... 51
11.3.3 Centrifuges 离心机........................ 52
11.4 Multi-Host Facilities 多主机设备............... 52
11.5 Non-product Contact Surfaces 非产品接触表面 52
11.5.1 Equipment for Buffers 缓冲液设备 52
11.5.2 Equipment for Media 培养基设备 53
11.5.3 Lyophilizers 冻干机........................ 53
11.5.4 Packaging Equipment 保证设备.. 54
11.6 Viruses, Mycoplasma and Prions 病毒和朊病毒，支原体 54
11.6.1 Control Steps 控制步骤............... 54
11.6.2 Control by Cleaning 清洁控制..... 54
11.6.3 Conclusion 结论........................... 55
11.7 Single-Use Equipment 独立使用的设备.. 55
11.8 Process Analytical Technology 工艺分析技术 56
11.8.1 PAT for Cleaning Process Control PAT对清洁过程控制 56
11.8.2 PAT Measurement Tools for Biotechnology Cleaning Processes PAT计量工具用于生物制品清洁工艺 57
11.8.3 Additional Considerations for PAT PAT的其他注意 57
11.9 Product Changeover 换生产品种............... 57
11.10 Clean Hold Considerations 清洁时限注意点 58

12.0 REGULATORY ISSUES 法规问题 60

13.0 REFERENCES 参考资料 62

14.0 SUGGESTED READING 建议阅读 64

15.0 APPENDIX – CARRYOVER CALCULATIONS 结余计算 65

16.0 LIST OF ACRONYMS 缩写清单 68

TABLES INDEX 目录

Table 3.2.1 **Cleaning Process Steps (Examples)** 清洁工艺步骤（举例） 7

Table 3.5.1 **Process Soil Categorization (Example)** 工艺用油（举例） 10

Table 3.5.2 **Surface Materials for Biopharmaceutical Production Processes** 11
用于生物制品工艺的表面材料

Table 5.1.2 **Comparison of CIP Grab Sampling versus Separate CIP Sampling Rinse**.................. 24
CIP简单取样 V.S. 单独的CIP淋洗水取样

Table 5.1.4 **Comparison of Swab Sampling and Rinse Sampling** 25
CIP擦拭取样 V.S. 单独的CIP淋洗水取样

Table 10.1 **CPP and CQA Considerations that have Potential Risk Impact to a Cleaning Process** 47
CPP和CAQ注意点对于清洁工艺的潜在风险

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.

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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

LD_{50}
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

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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.

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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

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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.

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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 reducing 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**.

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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
		Final sterile filtration equipment; product filling equipment
Buffer Preparation Soils	Aqueous salt solution without product	Buffer preparation equipment
		Buffer filtration equipment and hold tanks
Cleaning Agent Soils	Cleaning agent, including surfactant and alkali/acid	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.

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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.

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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.

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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

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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.

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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

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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

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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

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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 μS/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

than the carryover calculation. That default value is typically 10 ppm formulated cleaning agent. Note that the only difference between a carryover calculation for the active and a carryover calculation for the formulated cleaning agent is how the limit in the subsequent product is calculated – one uses a fraction of a dose and one uses a fraction of the LD_{50}. Once that calculation is performed, subsequent calculations for cleaning agents use the same formulas as for actives to determine the limit per swab or the limit per analytical sample.

For manufacturing of *bulk* actives, some of the same issues discussed for limits of actives also apply to cleaning agents. That is, a carryover calculation considering the total shared surface area results in extremely low limits, typically not measurable. However, the formulated detergents typically used may be removed by various purification processes (such as ultrafiltration or size exclusion chromatography) based on molecular weight. As with limits for actives, toxicity calculations may be applicable for downstream processes following the purification steps or for the last downstream process vessel.

4.3 Bioburden Limits

In considering bioburden limits following cleaning, it is not expected that the cleaning process itself results in sterile equipment. However, even if the process equipment is steamed in place or autoclaved prior to manufacture of the next product, it is typically the practice to evaluate bioburden to establish that the subsequent process is not overly challenged. Achievement of typical bioburden limits for non-sterile manufacturing (1-2 CFU/cm^2 for surface sampling methods) is considered more than adequate. For rinse sampling, some companies will utilize typical WFI values (10 CFU/100 mL), while others will utilize a value of either 100 CFU/100 mL or 1,000 CFU/100 mL. The rationale for the higher limit is that the equipment will be subsequently sterilized. Furthermore, the WFI value is the value for the WFI in the recirculating loop; once it is removed from that loop and placed in clean equipment, there is not *necessarily* an expectation that it will still meet the WFI value.

4.4 Endotoxin Limits

Endotoxin carryover to the final product is more of a concern after the various endotoxin removal steps. In those cases, endotoxin is typically measured only in the final rinse water, and limits are set at the typical WFI limit of 0.25 EU/mL. Prior to the endotoxin removal steps for cell culture processes, it can be expected that rinse samples should meet the WFI limit. For bacterial fermentation with *E. coli* (a gram-negative bacterium which will produce large amounts of endotoxin in the washing step), meeting the WFI limits following cleaning at the fermentation and harvesting steps may not be achievable. For that reason, some companies may not set endotoxin limits for cleaning for those steps, while others will set endotoxin limits but at a higher value, such as 5-25 EU/mL. Achieving such values indicates a measure of control in the cleaning process, and any possible carryover at those levels should be addressed by subsequent endotoxin removal process steps.

4.5 Visual Clean Criterion

The visual appearance of production surfaces is a direct measurement that verifies removal of residuals. Visual appearance is not a quantitative method but is very useful to directly verify that production surfaces are clean. Visual appearance is easy to perform provided there is ready access to the critical surfaces. It verifies the cleanliness of a significant area of production equipment. Literature indicates that low levels of residues (if present) are visible and can be detected. As used in biotechnology manufacturing, a visual clean criterion is typically used with swab and/or rinse testing

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for residues for cleaning validation protocols.

4.6 Modifying Limits

Manufacturers may establish action and/or alert *levels* on validated processes as part of routine monitoring. Those values are typically more stringent than the *pass/fail limits* in the validation protocol. Based on process capability showing consistently low values and the ability to maintain those values, manufacturers may perform a risk assessment and consider the use of more stringent limits for future validation protocols.

5.0 Sampling Methods

It is essential to a cleaning validation program that the *appropriate* sampling techniques are utilized. Sampling must be conducted with techniques appropriate for the equipment surfaces and for the nature of the study, including the analytical methods used. This section discusses types of sampling methods, sampling recovery validation studies, and the training and qualification of samplers.

5.1 Sampling Method Selection

Selection of a sampling method depends on the nature of the equipment and the nature of the residue being measured. Sampling methods discussed here are direct surface sampling, swabbing, rinse water sampling and placebo sampling. It should be noted that while regulatory documents refer to swabbing as "direct" sampling and to rinse water sampling as "indirect" sampling, it is preferable and more descriptive to refer to those sampling methods as "swab sampling" and "rinse sampling," and reserve the term "direct sampling" for techniques such as the use of visual inspection.

5.1.1 Direct Sampling Methods

Direct sampling methods (as used in this document) include visual inspection.

It is a well-accepted practice that a cleaning process should remove visible residues from the production process off of equipment surfaces. The visual inspection of equipment has limitations in that some equipment surfaces (e.g., piping) are usually not accessible for viewing. The use of optical equipment like mirrors or endoscopes, as well as the use of additional lighting, can help to facilitate visual inspection.

Remote inspection techniques (with fiber-optic probes and a LCD viewing screen) are utilized when visual inspection by a trained inspector is difficult to perform because of access to equipment surfaces, or when one prefers to supplement an "unaided" visual inspection procedure.

Borescopes, fiberscopes, and videoscopes allow visual inspection of hard-to-reach areas. Borescopes have been used to view the interior of piping and tank welds. Typical benefits of these scopes are that they: can fit into confined spaces not accessible to operators; are very maneuverable; have additional lighting attached; and may come with optional magnification and/or zooming capabilities. The major drawback of these scopes is the complexity of use, controlling lighting/brightness, and that the operator still has to make the determination if the area viewed is visually clean.

A remote visual camera allows operators to view remote areas on a screen. The camera has most of the same strengths and weaknesses as the scopes, with the added benefit that operators can typically also record video or take pictures. Multiple operators can, at the same time, view what is on the screen. The potential to record video and allow multiple operators to view the screen may help support a site's visual inspection training program. Pictures printed from the camera may distort the actual amount of residue present, since operators will typically zoom in on a particular area when taking a picture.

All these techniques, like visual inspection, require an adequate training program.

5.1.2 Rinse Sampling

Rinse sampling involves sampling the equipment by flowing water over all relevant equipment surfaces to remove residues, which are then measured in the rinse water. The most common rinse sampling technique is to take a grab sample from the final rinse water during the final rinse of the cleaning

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process. Another option is to fill the entire equipment with water after the cleaning procedure is completed. Then, a bulk sample is taken and analyzed. A third option is to utilize a separate CIP sampling rinse of defined volume following the completion of the final process rinse.

Advantages and disadvantages of both methods for CIP rinsing are shown in **Table 5.1.2**.

Table 5.1.2 Comparison of CIP Grab Sampling versus Separate CIP Sampling Rinse

	Grab Sampling from CIP Final Rinse	Separate CIP Sampling Rinse
Advantages	• Represents the normal cleaning process • Allows on-line testing • Requires no additional amounts of rinse water • Equipment can be used for further processing without additional steps	• Results can easily be used for carryover calculations • Represents what is left on surfaces after the completion of the cleaning process • More likely to result in an acceptable result if done correctly
Disadvantages	• Sample represents a worst-case carryover to the next batch (but can demonstrate robustness of the cleaning process) • Assumptions need to be made about sampling for carryover calculations	• Utilizes an additional step • Requires additional amounts of rinse water • Contamination is possible due to the method of water addition • Online testing not practical

A special case of rinse sampling is the sampling of small parts. Those parts may be sampled by swabbing, but there are two options for rinse sampling. One type of rinse sampling is extraction of the small parts. In an extraction procedure, the extraction solution (typically water for biotechnology residues) is placed in a clean vessel. The small part is then placed in the extraction solution and agitated or sonicated for a fixed time. The sampling solution is then analyzed for potential residues. A second type of rinse sampling for small parts is typically used for items with an orifice, such as filling needles. In this procedure, a fixed volume of sampling solution (again, typically water) is passed through the lumen and collected in a clean collection vessel. The sampling solution is agitated for uniformity, and then analyzed for the potential residues. Because the surface area and sampling volume are precisely known, limits can be accurately calculated for such situations.

5.1.3 Swab Sampling

Swab sampling involves wiping a surface with a fibrous material (most commonly). During the wiping procedure, the residue on the surface may be transferred to the fibrous material. The fibrous material is then placed in a solvent to transfer the residue to the solvent. The solvent is then analyzed for the residue by an acceptable analytical technique. The most common fibrous material is some kind of textile (knitted, woven or nonwoven) attached to a plastic handle.

In most cases the swabs are wetted with a solvent prior to sampling the surface. For TOC and conductivity, the solvent is almost always water. For sampling the same site, companies may choose to sample the same surface area with multiple swabs in order to provide a higher percent recovery of residue from the surface. In such cases, the additional swab(s) utilized may be either dry swab(s) or swab(s) wetted with the same solvent.

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5.1.4 Comparison of Swab and Rinse Sampling

Both swab sampling and rinse sampling are listed as acceptable sampling techniques in most regulatory documents. *(8,9,11)* Both methods have their advantages and disadvantages, as shown in **Table 5.1.4**.

Table 5.1.4 Comparison of Swab Sampling and Rinse Sampling

	Swab Sampling	Rinse Sampling
Advantages	• Enables the analysis of residues found on the specific surfaces. Includes the recovery of proteins that are denatured and/or adhered to the surface. • Allows for sampling of areas that are more difficult to clean (i.e., worst cases).	• During rinsing, the entire product-contacting surface is wetted. One analysis result represents the sum of all removed residues for the flow path. • The sampling procedure does not contaminate the equipment. Re-cleaning is not required after sampling. • This method allows for conclusions on the cleanliness of areas that are not accessible for swabbing.
Disadvantages	• Only discrete sampling areas can be analyzed, and these must represent the entire equipment; sampling must include worst-case locations. • The sampling itself can potentially contaminate the equipment. Re-cleaning may be required after sampling. • Some areas are not accessible for swabbing (e.g., piping systems).	• Only water soluble residues can be detected. • Those areas that are hard to clean cannot be identified. • Does not deal with residues that preferentially transfer from one part of the equipment to the next product. • May dilute out the residue to be undetectable by the analytical method.

In cases where the equipment surface is difficult to access for swabbing (e.g., piping), swabbing is not an option. It should be appropriately justified that the cleaning procedure is considered effective if swab testing will not be performed. The following situations will justify a decision to not swab a surface:

- Equipment not accessible for swabbing is constructed of the same materials as equipment that allows swabbing.
- Difficult to access equipment surfaces are exposed to the same residues and conditions as equipment surfaces that allow swabbing.
- Difficult to access equipment surfaces are cleaned with the same cleaning procedure (i.e., the same cleaning agents and the same temperature) as equipment that allows swabbing.
- The mechanical forces during cleaning in piping systems (e.g., turbulent flow) are higher compared to tank cleaning using spray balls.
- In contrast to tanks, the piping system is completely filled with flowing liquid during cleaning.
- Rinse sampling appropriately addresses the issue of cross-contamination from those surfaces.

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5.2 Placebo Sampling

In biotechnology, placebo sampling generally does not include actual product placebo; instead, it includes only WFI or the aqueous processing buffer without any product. In this sampling process, the equipment is cleaned. Following cleaning, a manufacturing process is performed (to the extent feasible) using only WFI or buffer. Following processing, the WFI or buffer is evaluated as any other cleaning validation sample, typically for TOC (or Total Protein), conductivity, bioburden and endotoxin, as measures of possible contamination of a manufactured product with those residues. Placebo runs can be done for both bulk and formulation/fill manufacturing to demonstrate actual carryover to the processed material.

5.3 Sampling for Microbial and Endotoxin Analysis

Sampling for bioburden involves rinse water sampling, swabbing or contact plates. Rinse water sampling for bioburden must involve the use of sterile sample containers. A careful sampling technique is required for any microbial method to avoid external contamination of the sample.

Sampling for endotoxin is almost always a rinse water sample.

There is nothing unique to biotechnology about the use of these sampling techniques for microbial evaluation.

5.4 Sampling Recovery Studies

Sampling recovery studies are generally required to adequately demonstrate that a residue, if present on equipment surfaces, can be adequately measured or quantified by the combination of the analytical method and the sampling procedure. These studies provide a scientific basis for utilizing those sampling and analytical methods to measure residues. Three types of sampling recoveries are discussed below: swab sampling recovery, rinse sampling recovery, and "visual examination" recovery. For swab and rinse sampling, recovery studies may be performed as part of the analytical method validation, or they may be performed as separate studies, once it is determined that the analytical method can appropriately measure residues in solutions. Sampling recovery studies are laboratory studies involving coupons of sampled equipment of different materials of construction (such as stainless steel, glass, PTFE and silicone) spiked with residues to be measured.

5.4.1 General Considerations

Recovery studies may not be required for certain residues which are known to be readily water soluble and are used well below the solubility limit, such as sodium hydroxide or phosphoric acid used as cleaning agents.

In performing recovery studies for swabbing and rinse sampling, the amount of material spiked onto coupons should represent an amount equal to what could be present at the residue limit, as this represents a worst case.

The residue spiked should be the same residue present at the end of the cleaning process. For biotechnology protein actives, this is actually degraded protein fragment. However, it is common practice to spike the native protein active, as this is simpler and represents a worst case. For bulk biotechnology manufacturing, some manufacturers only perform recovery with the bulk active, whereas others will also utilize an early stage harvest product to represent early stage residues.

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Recovery values should be established for all surfaces sampled. For swab and rinse sampling, this may be accomplished by performing recovery studies on all surfaces. An alternative is to perform one residue study on a surface, which through documented evidence, is equivalent (in terms of percent recovery) to other surfaces for which a formal recovery study is not performed. This is essentially a grouping or family approach for recovery studies. Equivalence for establishing the group or family may be established based on published studies or in-house data. Another approach used by some companies is to exclude formal recovery studies for sampled surfaces constituting less than a small percentage (such as 1% or 2%) of the total equipment surface area; in such cases, the recovery value used for that excluded surface is the lowest recovery of any other surface type for which a formal sampling recovery study was performed, or the minimum acceptable recovery percentage required by the company's procedures.

Swab recovery studies are typically performed on a nominal coupon square surface area of either 25 cm^2 or 100 cm^2. In sampling manufacturing equipment for a protocol, it is not always possible to swab a 10 cm X 10 cm area (it might be necessary to swab a 5 cm X 20 cm area). Furthermore, it might not be practical to swab exactly 100 cm^2 (an area of 60 cm^2 or 128 cm^2 may be required because of the specific equipment geometry). In such cases, the recovery percentage based on sampling 10 cm X 10 cm may be applied to each of those cases. If such an approach is used, a range of acceptable surface area (such as 25% to 150% of the nominal sampled area) should be established. However, if the sampled area for equipment surfaces in a protocol varies from the nominal value, the residue limit for that sample should be adjusted based on the actual surface area swabbed.

5.4.2 Swab Recovery

For swab recovery studies, coupons are spiked with solutions of the target residue, allowed to dry, and sampled with the swabbing procedure to be utilized in the cleaning validation protocol. The swab is desorbed in a suitable solvent, and the amount of residue is measured in that solvent sample. The amount recovered is compared to the amount spiked on the coupon, and the result is expressed as percent recovery. Because swabbing is a manual procedure, typically each person performing a recovery study performs three replicates. It is preferable to have at least two persons perform swabbing recovery studies for each combination of residue and surface type. The recovery percentage established by the study may be defined in different ways, but typically is defined as the lowest average recovery of any one analyst. An acceptable swab recovery depends on how that swab recovery is being used. If the recovery is performed to qualify the sampling method *without correction* of either a limit or an analytical result, a recovery of 70% or more is typically required. If the recovery percentage is *used to correct* a residue limit or an analytical result, a recovery of 50% or more is typically required. An upper limit for percent recovery should be established.

At a minimum, recovery values are generally performed at the residue limit on the surface (in $\mu g/cm^2$, for example). While it is possible to perform recoveries at different spiked levels, in general, there is little value to such additional spiked levels because of the variability of the sampling procedure. It is preferable to perform additional replicates at the one-residue limit rather than studies at additional levels. Acceptable variation for recovery results at one spiked level is typically on the order of 15-30% RSD.

5.4.3 Rinse Recovery

Rinse recovery studies address the validity of rinse sampling for that residue. They demonstrate that if the residue were on a surface, that residue would be effectively removed and could be analyzed in the rinse solution. Rinse recovery studies address the U.S. FDA's "dirty pot" and "baby/bath water"

analogies. *(8)* Rinse recovery studies, like swab recovery studies, are performed on coupons that have been spiked with solutions of the target residue and then allowed to dry. For swab recoveries, it is necessary to perform the exact swabbing procedure to be used in the cleaning validation protocol. For rinse sampling, in contrast, the exact rinsing procedure cannot be *duplicated* in the laboratory. However, it is possible to *simulate* the rinsing procedure in the laboratory. Where possible, the conditions of the simulated rinse should be the same as the equipment rinsing situation. This includes the selection of rinsing solvent (typically water), as well as the temperature of the rinsing solvent. In other cases, the rinsing conditions should be selected as the same or worst case as compared to the equipment rinsing situation. For example, the ratio of solvent to sampled surface area should be the same or lower in the recovery study compared to the equipment-rinsing situation.

One method of simulating the rinse process is to suspend a spiked coupon above a clean collection vessel and cascade the rinse solution across the surface into the collection vessel. Another method is to spike the bottom of a beaker of the appropriate material of construction, allow the residue to dry, add rinse solution to the beaker and apply gentle agitation for a time which approximates the time of the final rinse. The rinse solution is either pipetted or decanted from the beaker and analyzed. A third option, used in cases where a beaker of suitable material of construction is not available, is to place a spiked coupon in the bottom of a beaker and perform a simulated rinse, as in the second situation.

Since rinse sampling is not significantly operator dependent, three replicates by one operator are adequate to determine percent recovery. Acceptable percent recoveries are typically established at the same levels and conditions as for swab recovery studies.

5.4.4 "Recovery" in Visual Inspection

This process is actually the determination of a quantitative "visual detection limit" where visual examination is the sole sampling/analytical method and "visually clean" is used as the sole acceptance criterion for the given residue in the absence of swab or rinse sampling for that residue. ***(12,13)*** If visual examination is used to supplement swab or rinse sampling, such determination of a visual detection limit is not required. A visual detection limit under specified viewing conditions can be determined by spiking coupons of the equipment surface materials with solutions of the residue at different levels (in $\mu g/cm^2$) and by having a panel of trained observers determine the lowest level at which residues are clearly visible across the spiked surface. The significance of such a visual detection limit is that if equipment surfaces are determined to be visually clean under the same (or more stringent) viewing conditions in a cleaning validation protocol, the level of the residue is below the visual detection limit. Appropriate viewing conditions include distance, lighting and angle. The visual limit depends on the nature of the residue as well as the nature of the surface (for example, stainless steel vs. PTFE) and the visual acuity of the inspector. Typical values reported in the literature for a visual detection limit are 1-4 $\mu g/cm^2$. ***(14)***

5.4.5 Recovery for Bioburden and Endotoxin Sampling

Recovery studies to determine percentage recovery *from surfaces* are not appropriate and are not required for microbiological sampling or for endotoxin sampling for the following reasons:

1. The question of enumeration in microbiological tests—"colony forming units" are typically counted, as opposed to individual organisms.
2. Vegetative organisms will die or lose viability when dried on a coupon in a standard sampling recovery procedure.
3. It is unclear which species should be used for a recovery study.

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4. The limits set for bioburden typically are significantly below what could possibly cause either product quality issues or process performance (e.g., SIP) issues; therefore, even though recovery may be low (<50%), product quality and/or process performance are not impacted by excluding a recovery factor.

5.5 Training and Qualification of Samplers

Training involves the steps taken to assist the prospective sampler in learning the technique of sampling/inspection. For purposes of this section, "sampling" and "sampler" also include "inspection" and "inspector" for visual evaluation. Qualification involves the process of "certifying" that the prospective sampler can appropriately sample.

Training always precedes qualification. At a minimum, the trainee should read the sampling procedure, and a trained sampler should demonstrate the correct procedure. During the reading and demonstration, the trained sampler provides commentary on the rationale for certain practices or aspects of the sampling procedure. Demonstration of technique may also utilize a visual indicator on the swabbed surface, which assists the trainee in seeing consequences of poor technique. The last step in training is demonstration of the correct procedure by the prospective sampler.

Qualification processes used for sampling will depend on the type of sampling performed. Qualification may involve merely demonstration of the correct technique (that is, the last step of the training process), or it may involve a "test" which challenges the trainee's ability to perform the activity correctly (e.g., perform a visual inspection using an array of coupons, including some that are soiled and others that are not, or perform a swab sampling for a known soil residue level on coupons). Either type of qualification may be repeated on a regular basis, or upon any retraining of a sampler.

5.5.1 Key Issues for Training for Swab Sampling

It is preferred to have a separate swab sampling SOP for training prospective samplers. This helps prevent "procedure creep" which might occur if the swabbing procedure text is just repeated in every protocol. It also helps ensure that the same sampling procedure is used in recovery studies as in protocol execution, and thus simplifies training.

Four keys to consistency in swab sampling training are:

1. Consistency of wetting the swab head
2. Consistency of the swabbing motion (including overlapping strokes)
3. Consistency in applying pressure
4. Consistency in swabbing of the correct surface area

It is assumed, of course, that the correct swab, the correct number of swabs, and the correct wetting solution (if any) for the swab are utilized. A fifth key factor for swab sampling involving TOC is emphasis on preventing external contamination of the swab due to the ubiquitous presence of organic carbon. It can be beneficial to emphasize "aseptic technique" used in microbiological sampling in training swab samplers, particularly when the sampling involves TOC analysis. In contrast to aseptic techniques used in microbiological sampling, however, the negative consequences (that is, artificially high TOC values) of using isopropanol on gloves or on adjacent surfaces prior to sampling should be emphasized.

Since swab sampling is not unlike manual cleaning processes, in that it is highly dependant on a person for consistency, consideration should be given to retraining and/or requalifying swab samplers on an established basis. Retraining may involve the same process as for initial training, or may involve only portions of that initial training. Requalification generally involves a repeat of the initial qualification

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process. The need for retraining and/or requalification should also be addressed as part of change control for the swabbing procedure and as remedial action when swab sampling "operator error" is suspected in the investigation of a non-conforming result.

5.5.2 Key Issues for Training for Rinse Sampling

It is preferred to have a separate rinse sampling SOP for training prospective samplers. For CIP systems, the rinse sampling procedure may be the same procedure that is used for sampling water systems, appropriately modified to cover sampling of process equipment.

The major concern for accuracy in rinse samples is to prevent contamination of the rinse sample due to the sampling port and the environment around the sampling port. This includes adequately flushing or cleaning the port prior to taking a sample, as well as avoiding sample contamination due to the use of isopropanol on gloves or the use of isopropanol to clean the port (prior to sampling). As in swab sampling training, it can be beneficial to emphasize the "aseptic technique" used in microbiological sampling in training rinse samplers, particularly when the sampling involves TOC analysis. In training rinse samplers to take a sample for the final rinse of a CIP cycle, timing of the sampling process is critical. Typically the very last portion of the rinse is sampled; but, it may be acceptable to sample before that time if such sampling represents a worst case. However, once process rinsing is complete, there is no way to go back and collect a rinse sample (unless a separate sampling rinse is performed).

Since the consistency of rinse sampling is less operator dependent, the need for retraining and requalification should also be addressed as part of change control for the rinse sampling procedure, as well as when rinse sampling "operator error" is suspected in the investigation of a non-conforming result.

5.5.3 Training for Visual Inspection

Training for visual inspection depends on whether the visual inspection is part of a protocol execution or whether the visual inspection is the laboratory "limit of detection" determination. In either case, it is preferred to have a visual inspection SOP, so that training can be for that SOP. Visual inspectors for either type of visual examination should have appropriate vision tests

For training of visual inspectors in a *protocol execution*, key issues are:

- Access to sites for viewing
- Appropriate lighting
- Ability to discern the difference between residues on the surface and surface imperfections

An important element of visual inspection training is knowing when to call for further analysis to determine the nature of the residue. For example, if what appears to be rouge is seen on the equipment, the presence of that residue should be noted. Determining whether that residue causes a failure in the cleaning process is a *separate* decision.

The procedure for visual inspection for laboratory "limit of detection" determination is generally different from that of visual inspection during protocol execution, because the objective is different. The objective is to determine at what level a certain residue can be consistently seen across a spiked surface in order to correlate a visual detectability limit with a level of known residue(s) below that spiked level. This procedure may be in a separate SOP, or may be incorporated in an overall SOP for visual inspection. In addition to the same elements that are included in training for protocol execution, a key for training in this procedure, which involves viewing spiked coupons, is a careful distinction between a visually clean surface, a partially soiled surface (in which residue is apparent only over a portion of the spiked area), and a "fully" soiled surface.

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6.0 Analytical Methods

It is essential to a cleaning validation program that the appropriate analytical methods are utilized. Analytical methods must be appropriate in that they can adequately detect the residue(s) of concern. It is also important to understand what can be concluded from the analytical result (e.g., was the product not removed or was the cleaning agent not removed?). The results of testing will determine if the cleaning validation cycle is acceptable or if it needs to be redeveloped. Thus, it is important to have confidence in the results. This section discusses how to select the appropriate assay methods, detailed information on the applicability and use of nonspecific assays and microbial test methods, and assay method validation.

6.1 Specific Analytical Methods

Specific analytical methods are those which measure a certain residue in the presence of *expected* interferences. In a cleaning process for biotechnology products where the specific analyte is the active protein, such interferences may include degradation products and related substances, excipients, cleaning agents and cleaning agent by-products. Examples of specific methods include HPLC, ELISA, SDS PAGE, and PCR. Each of these methods requires the use of an appropriate reference standard. In contrast, nonspecific analytical methods measure a general property, such as conductivity or TOC, which could be due to a *variety* of analytes or sources.

Selection of an analytical method will depend on the nature of the residue as it exists after the cleaning process. Only if a protein (or other organic active) is *not degraded* during the cleaning process (surviving high temperatures and pH extremes in an aqueous environment, for example) does it make sense to use a specific analytical method for that active. The advantage of using a specific analytical method in this situation is that it gives a precise measure of the major residue of concern – the active itself.

If a specific analytical method for an active protein were utilized following a cleaning process which has been demonstrated to denature (degrade) that active protein, it is likely that residues of the active protein would be non-detectable (i.e., not measurable) by that specific analytical method. Residues of that protein would be various degraded fragments. If the native protein were actually detected using a specific method for that protein, it is likely that there had been a serious problem with the cleaning process, such as a clogged spray device causing a lack of coverage of that portion of the equipment surface. In such a case, failure would also most likely be detected by a nonspecific method and/or by visual examination. Consequently, if a specific assay method is used, a nonspecific assay method is also required, unless studies prove that the product is not degraded by the cleaning process.

In biotechnology cleaning validation, specific analytical techniques such as HPLC are more likely to be used for detergents, because the surfactants or other functional materials in the detergents are not likely to degrade in the cleaning process. However, it should be noted that nonspecific methods can also be used for detergents and other cleaning agents.

6.2 Impact of Inactivation/Degradation of the Active

Product inactivation means that the active protein is modified in some way such that it is no longer active and may no longer be measurable by *specific* analytical methods for that native protein. This modification usually involves degradation of the active protein into smaller fragments, but may also involve a process in which larger molecules are formed. A key issue for process equipment cleaning in biotechnology manufacturing is the degradation or deactivation of the active protein during the cleaning process. This is a result of cleaning processes in biotechnology utilizing hot, aqueous, alkaline and acidic cleaning solutions. Under such conditions, it is well recognized that protein actives

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will degrade. This degradation affects several issues in the cleaning and cleaning validation process. Because of the degradation, the residues of the active protein (which are actually now residues of the *degraded* active protein) are more readily rinsed away during the rinsing step of the cleaning process. This is because the degraded fragments typically have a lower molecular weight and are potentially more polar, both conditions leading to greater water solubility. A second consequence of the degradation is that it no longer is scientifically justified to have an analytical method which is *specific* for the native protein. For this reason, a nonspecific method such as TOC or Total Protein is typically used to measure residues of the degraded active (as well as other organic molecules) in a cleaning validation protocol. A third consequence is that limits in bulk biotechnology manufacturing are typically *not* appropriately established based on a "fraction of a dose" calculation of the native protein, since the residues are degraded fragments. Since residues being sampled are residues of the degraded protein, it may also make more scientific sense to perform sampling recovery studies based on recovery of the degraded fragments. However, assuming an increase in solubility for degraded proteins, sampling recovery studies on the native protein will typically be a worst case as compared to recovery of degraded fragments.

While it is assumed in almost all cases that the active proteins or other large organic molecules produced in biotechnology manufacturing are readily degraded in hot, aqueous alkaline conditions, it is desirable to demonstrate this with a laboratory study. In such a simple "beaker" study, the bulk active protein is exposed to the conditions of the cleaning process, including cleaning agent concentration, temperature and time. At the end of that exposure time, the pH is neutralized, and the temperature is reduced. The resultant solution is then analyzed for the active protein by the specific analytical procedure (such as ELISA, HPLC or a bioactivity assay). In such a procedure, the ratio of protein to cleaning solution should represent the same ratio present during cleaning, or a worst-case ratio (a worst-case is a higher ratio of protein to cleaning solution).

The assay methodology for such studies must be appropriate and valid. Care needs to be exercised in performing such a study to ensure that the chemicals in the cleaning solution do not interfere with the analytical procedure. This can be addressed by having adequate controls, such as adding the active to a solution of the neutralized cleaning solution at ambient temperature. If chemicals in the cleaning solution interfere with the specific analytical procedure, another option is to remove them by diafiltration. If it is just the surfactants in the cleaning agent that interfere, another option is to perform the degradation study with just the equivalent amount of alkali present in the cleaning solution. Note that in many cases, cleaning in a biotechnology facility utilizes alkaline cleaning agents *followed* by an acidic cleaning solution. Current evidence suggests that it is the alkaline portion that is most effective in degrading active proteins. ***(15)*** Companies may choose to perform a degradation study only with the alkaline agent and not pursue degradation studies with the acidic solution unless the alkaline cleaning agent alone is inadequate for degradation.

6.3 Nonspecific Analytical Methods

6.3.1 Total Organic Carbon (TOC)

Most of the compounds used in biotechnology processes are of organic nature. TOC can detect organic carbon with a good sensitivity in the sub-ppm range; however this sensitivity may still not be adequate for highly active substances. The method can be semi-automated with an autosampler and has a short analyzing time. In contrast to specific analytical methods, TOC analyzers can detect all organic residues, including complex mixtures of compounds like cell culture media or product degraded by the cleaning process.

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With TOC, it is not possible to distinguish between a biotechnology product and other organic compounds present in the same sample. As a consequence, all organic carbon is assumed to be product, representing a worst-case approach. Another aspect is the potential for sample contamination with organic substances during sampling and testing, requiring well- trained personnel and clear sampling instructions. Special care should be taken to ensure that the sampling container does not introduce unacceptably high amounts of carbon to the sample.

Different TOC analyzers are commercially available. All instruments oxidize organic carbon and measure the resulting carbon dioxide. When selecting a TOC instrument, care should be taken to select an instrument and instrument parameters that are able to completely oxidize the organic carbon present.

The TOC method can be used for rinse and swab measurements. If used for final rinse water testing, samples can be analyzed directly. If used for swab testing, the organic carbon has to be extracted from the swab after sampling. It is important to consider some additional topics during TOC swab method development, such as swab material and technique. Swabs should not significantly contribute carbon to the sample and should not adsorb significant amounts of the residue such that it is not released for analysis. The sampling technique (e.g., swab size and shape, swabbing pattern, swab container and extraction method) is much more complex in comparison to rinse water testing. The swabbing technique can have a high influence on residue recovery.

6.3.2 Total Protein

Several total protein assays of different sensitivity are commercially available. Assays often used are Bradford, Lowry or BCA. Total protein assays are not product specific, but specific towards a class of molecules. Total protein assays can be used if the majority of the residues are proteins. If proteins are just one of many residues present (e.g., cell culture fermentation), the use of an assay with a broader spectrum (e.g., TOC) should be considered.

One advantage of total protein assays is potential commercial availability. Different companies offer test kits and support during test implementation. Lead times for implementing commercial test kits are typically shorter compared to in-house developed methods. In-house methods may be developed to provide enhanced sensitivity.

Proteins often degrade (e.g., by hydrolysis during a cleaning cycle if high pH and temperatures are used). During assay implementation, it should be investigated if the assay still can detect protein after exposure to cleaning agents.

6.3.3 Conductivity

Conductivity measurement is a very sensitive method to detect dissociated ionic substances in water samples. WFI has a conductivity of ≤ 2.4 µS/cm at 65°C. For cleaning validation purposes, conductivity readings are expressed in milli-Siemens/cm (mS/cm) for higher concentrations (such as cleaning solutions) and micro-Siemens/cm (µS/cm) for lower concentrations (such as final rinse waters). It is often used to measure cleaning agent residues (e.g., caustic agents) and to control automated cleaning processes (e.g., CIP). Conductivity instruments can be used for a wide range of concentrations by exchange of conductivity probes. Conductivity readings are highly influenced by the sample temperature. Either temperature adjustment of the sample or automated temperature compensation can be used to standardize the measurements.

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Conductivity is a nonspecific method that correlates linearly (within a defined range) to the ion concentration in an aqueous sample. Analytical instruments are robust and can be used on the manufacturing floor by trained personnel. The high influence of the sample temperature on the instrument reading should be considered to avoid incorrect results. The method cannot differentiate between different ions. Therefore, as for TOC, all conductivity results above the water baseline should be attributed to the contaminant in question (e.g., the cleaning agent). For biotechnology cleaning validation applications, conductivity is normally not used to detect product residues.

To allow correlation of conductivity readings with concentrations of cleaning agents, a dilution curve (conductivity vs. concentration) should be established (at a relevant temperature) by conductivity measurements of different dilutions in the relevant range near the acceptance value.

6.3.4 Visual Inspection

Visual inspection is a qualitative method to determine cleanliness on specific equipment surfaces. Visual inspection has been demonstrated to be a simple and effective direct sampling method in the evaluation of equipment cleanliness.

Visual inspection does have multiple weaknesses that are inherent. Extensive training and a detailed documented procedure are required to ensure that "visually clean" from one operator to the next is consistent. What one can visually see will vary with distance, angle, lighting, the nature of the surface and inspector's visual acuity. Some equipment surfaces (e.g., piping) are usually not accessible for visual inspection. The use of optical equipment (e.g., mirrors, remote visual cameras or endoscopes) can help to facilitate visual inspection. In order to view some equipment areas, wear and tear on the equipment may occur (e.g., the disk stacks in a centrifuge are not designed to be removed and inspected for visual cleanliness after cleaning). In other cases, an operator may be required to enter a confined space for viewing equipment surfaces.

The visual inspection procedure should specify how operators are to deal with visual observations. Visual inspection could find four different types of visual observations: residue, surface anomalies, foreign object and water pooling. Residue is the *main* concern, which would constitute a visual failure when one is looking at the acceptability of a cleaning cycle. A sample of the residue should be collected for further testing, if possible, to assist in the investigation of the cause. Typically, surface anomalies and foreign objects are not considered visual inspection failures for cleaning validation purposes, but must be further investigated and corrected, as applicable. Surface anomalies should be noted and a "suitability for use" assessment should be performed to remediate any issue(s) found. Rouge is the most common type of surface anomaly discovered during visual inspection; rouge is generally considered a preventive maintenance problem, not a cleaning process problem. Foreign objects and their removal should be noted. How the foreign object came to be in the equipment should also be investigated. Water pooling should be documented, and the cause should be investigated.

All equipment surfaces that can be readily inspected visually should be visually inspected. Visual inspection may not be performed on the interior of lines and tubing (although outlets may be inspected) on equipment where disassembly of the equipment is not practical or possible, or where inspection of the equipment could potentially be dangerous to the inspector (e.g., entry into a confined space).

A visual inspection training program should be developed for visual inspection. Inspectors typically should be trained and/or requalified on an established basis. If visual inspection is not possible on an area of concern, it is important to ensure that other sampling methods (such as rinse sampling) can adequately detect potential residues of concern.

The use of "visually clean" alone (in the absence of other analytical methods such as TOC or conductivity) is not generally used in the biotechnology industry, because all critical surfaces are not readily available for visual examination.

6.4 Microbial Test Methods

The U.S. FDA's cleaning validation guidance states that "Control of the bioburden through adequate cleaning and storage of equipment is important to ensure that subsequent sterilization or sanitization procedures achieve the necessary assurance of sterility." *(8)* Endotoxin is a concern in that steam sterilization does not destroy or remove endotoxin. Thus, both bioburden and endotoxin are typically monitored and controlled during the manufacturing and cleaning processes. Typically microbiology sampling is performed during all cleaning validation studies throughout the manufacturing process.

6.4.1 Endotoxin

Typically, endotoxin testing is performed for cleaning validation runs. Endotoxin testing may not need to be tested in upstream cell culture and initial purification processes where it is proven during process validation that one or more purification steps is able to effectively remove endotoxin that is present. Typically, a three logs or greater reduction of endotoxin in endotoxin removal steps is required to justify decisions not to test for endotoxin upstream. Endotoxin methods are typically compendia methods.

6.4.2 Bioburden

Testing of bioburden is typically done through rinse water sampling, although other methods may be used. The benefit of rinse water sampling for bioburden is that it is convenient. Typically, rinse water sampling is being performed to verify removal of protein and cleaning agent(s), so one additional rinse water sample does not require significantly more work. Also, bioburden testing of rinse water is typically already a qualified method for testing water systems for bioburden. The biggest weakness of rinse water sampling is that the full range of the acceptance criteria is not able to be utilized, for example, if 100 ml of rinse water is used for testing with an acceptance criterion of 10 CFU/mL. The typical number of colonies that can be counted is 300 before TNTC (Too Numerous To Count) is achieved; this only allows an acceptance criterion of 3 CFU/mL before failing to meet the acceptance criteria. In most situations this is not an issue; it may result in the need to test smaller sample volumes. If this situation occurs, it is important to test an adequate amount of rinse to ensure that bioburden is detected.

Two methods for measuring directly on surfaces are swab and contact plate method. For swab samples, the swab can be desorbed, and a count can be made by a pour plate method. Contact plates are directly incubated and enumerated. The biggest concern with contact plates and swab procedures is potentially exposing product contact surfaces to an unknown media or buffer solution from swabs; thus, acceptable removal of this media or buffer solution should be demonstrated before manufacturing can occur. Another concern is that contact plates require flat surfaces.

6.5 Analytical Method Validation

This section focuses on analytical method validation for "chemical" residues. Typically, endotoxin methods are compendia methods and do not require formal validation but require a confirmation for their application of use or suitability. Microbiological methods that are approved microbiology laboratory methods do not require additional method validation.

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6.5.1 General Principles

Since one key part of cleaning validation is setting residue limits and then measuring (using an analytical method) the actual residues left on surfaces after cleaning, it is critical that the analytical method be appropriately validated. Method validation is typically accomplished using the criteria in ICH Q2(R1), *Validation of Analytical Procedures: Text and Methodology*. ***(16)*** However, the types of assays listed in ICH Q2 do not explicitly cover cleaning validation methods. Some companies will essentially validate analytical methods much like an "assay" in ICH Q2, establishing accuracy, precision specificity, linearity and range, with the added determination of LOD/LOQ. LOD/LOQ must be below the acceptance limit for the sample and ideally is significantly below the acceptance limit so that the robustness of the cleaning process can be established. In addition to the ICH Q2 parameters, sample stability as a function of storage conditions (time, temperature, vial, etc.) may be evaluated if there is a significant interval between sampling and analysis.

In cases where a nonspecific method (such as TOC) is utilized, it is not necessary to compensate for the lack of specificity by "other supporting analytical procedures" (as suggested in ICH Q2). The reason for this is that for cleaning validation purposes, the limit value is not a target (as it is for a potency assay); rather the limit is a value *not to be exceeded*. As long as other organic substances contribute positively to the TOC value, and as long as all measured carbon is attributed to the target residue, such complementary methods suggested by ICH Q2 are not required. Furthermore, it is not required to correlate TOC results with a specific analytical method, except to the extent that accuracy in method validation is established using a known standard that establishes the concentration or activity by a specific analytical method. While Detection Limit and Quantitation Limit are not part of the "Assay" requirement in ICH Q2, it is critical that these values be at or below the preestablished limit for the residue (otherwise it would not be possible to claim that residues were below the predetermined limit values). However, it is not necessary to drive detection or quantitation limits as low as possible; having detection or quantitation limits around 10% of the residue limit in the analytical sample is ideal (but not always possible) to establish the robustness of the cleaning process. Assay capability should take into account both the target/limit and the process capability and provide relevant measurements for both.

When performing carryover calculations (as is typically done for the formulation/fill side of biotechnology manufacturing) it should be ensured that the analytical methods that will be used for cleaning validation are sensitive enough to meet the acceptance criteria. To provide reliable results for carryover calculations, the results should be equal to or above the LOQ. Results between the LOQ and the LOD typically show a higher-than-acceptable variation of the results obtained and are typically reported as less than LOQ.

For companies that use a pass/fail analytical method for meeting cleaning validation limits, analytical method validation is less extensive. In such a procedure, the only conclusion of the analytical procedure is whether the experimental sample is less than or equal to the pass/fail value, or above that pass/fail value. Accuracy and precision are typically performed only at the residue limit, but linearity and range are not performed. Note that in this case, the pass/fail value selected should take into consideration any applicable correction factor due to the sampling method, recovering less than 100% from the surface. Pass/fail analytical procedures are more likely to be part of a cleaning verification mode, used in the manufacture of early clinical trial materials.

Analytical method validation protocols may only include validation of the residue in solutions. They may also include sampling recovery studies, although those sampling recovery studies may be performed as separate studies apart from the analytical method validation.

Acceptability of the variability of results for parameters such as accuracy and precision for methods

at typical residue levels are generally much broader than in a typical potency assay. RSD requirements of 15-20% are typical.

6.5.2 Compendia Methods

Compendia methods do not require separate analytical method validation, provided those methods are used within the parameters in the compendia. For example, a compendia method for endotoxin is generally appropriate for measuring endotoxin in final rinse water samples.

When using TOC in rinse water samples (a compendia method), additional work should be done to support the applicability of that method to test samples that could have TOC values above 500 ppb, or where a linear range is to be established. Just performing system suitability as specified in the various pharmacopeias may not be adequate to demonstrate that the analytical procedure could accurately analyze samples at 1 ppm or 5 ppm. For that reason, analytical method validation as for any other method should be considered. An additional reason for formal method validation for TOC in rinse water samples is that the compendia methods are essentially set up as a pass/fail test, not as a quantitative assay.

Measurement of TOC in swab samples does not follow a compendia method and must be validated prior to use in cleaning validation or verification studies. Particular attention should be given to the choice of swab, swabbing technique, and recovery of residue from the swab (see **Section 6.3.1**).

6.5.3 Visual Inspection

Method validation in this case is actually the determination of a quantitative "visual detection limit" in cases when visual examination is the sole sampling/analytical method and "visually clean" is used as the *sole acceptance criterion* for the given residue in the *absence* of swab or rinse sampling for that residue. If visual examination is used to supplement swab or rinse sampling, such determination of a visual detection limit is not required. A visual detection limit *under specified viewing conditions* can be determined by spiking coupons of the equipment surface materials with solutions of the residue at different levels (in $\mu g/cm^2$) and by having a panel of trained observers determine the lowest level at which residues are clearly visible across the spiked surface. The significance of such a visual detection limit is that if equipment surfaces are determined to be visually clean under the same (or more stringent) viewing conditions in a cleaning validation protocol, the level of the residue is below the visual detection limit. Appropriate viewing conditions include distance, lighting and angle. The visual limit depends on the nature of the residue as well as the nature of the surface (for example, stainless steel vs. PTFE).

6.5.4 Bioburden Methods

Approved and qualified microbiological lab procedures do not require additional method validation for use in cleaning validation programs.

6.5.5 Use of a Contract Laboratory

Contract laboratories can be used to develop and validate an analytical method for use in cleaning validation. The same considerations given to method validation discussed in **Section 6.5.1** apply in this situation. However, if the method is to then be performed by the biotechnology company, it is mandatory to have a method transfer protocol established and executed so the method can be used

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"in house," If the method is developed by a contract laboratory and protocol samples are analyzed by that contract laboratory, no transfer protocol is required. It is preferable that analytical method validation protocol be reviewed and approved by the biotechnology company prior to execution of that protocol. If an analytical method has been developed and validated *previously* by the contract laboratory, the biotechnology company must review that protocol and the final report to determine the acceptability of the method for its (new) intended use. If an analytical method has been developed and validated by a biotechnology company and cleaning validation samples are to be analyzed by a contract laboratory, a method transfer protocol must be established to determine that the contract laboratory can suitably analyze samples using that method.

7.0 Cleaning Validation Protocols

Cleaning validation protocols have many of the same elements as process validation protocols. For reasons of clarity, the format of a cleaning validation protocol usually follows the same approach (as appropriate) as used for process validation protocols for a given company. Common elements include purpose, scope, responsibilities, applicable product(s) and equipment, cleaning SOP, acceptance criteria and a requirement for a final report. Key elements for cleaning validation protocols include residue limits (see **Section 4.0**), sampling procedures (see **Section 5.0**) and analytical methods (see **Section 6.0**). The organization and rationale for cleaning validation protocols for biotechnology manufacturers is fundamentally the same as for other pharmaceutical manufacturers.

7.1 Cleaning Verification Protocols

Protocols for *cleaning verification* purposes are the same as for cleaning validation, except that the protocol is specific to one cleaning event. From a compliance perspective, the protocol applies only to the one cleaning event (although from a scientific perspective the data may suggest similar performance if the cleaning event were repeated). Another difference is that because a verification protocol is typically performed on a unique cleaning event, there may be limited cleaning development before execution of that protocol. Alternatively, companies might use a concept that defines explicit requirements for cleaning verification in an SOP and documents the specific activities, sample positions, etc., on a form, which will be approved.

7.2 Key Issues Based on Regulatory Changes

It is assumed that the validation protocol is not written and approved until the cleaning process has been designed and developed (see **Section 3.0**). This is particularly important as it relates to a life cycle approach to validation. Two key issues for protocols, each of which is in a state of flux because of regulatory changes, are discussed below.

7.2.1 Number of Runs in a Protocol

The traditional approach for cleaning validation protocols has been to require an evaluation of a minimum of three consecutive runs of the cleaning processes. By consecutive, it has meant that no cleaning events of that same process are skipped without appropriate rationale.

This practice is in flux because of changes in approach by the U.S. FDA; the Agency no longer suggests a minimum of three runs. ***(17,18)*** Rather, the manufacturer must provide a rationale (based on its understanding of the process) for determining the number of runs. Providing such a rationale is not straightforward for cleaning processes, and some companies specify in their master plans that three runs will be required unless there is a written rationale for a different number. It should be noted that as of publication of this Technical Report, the question of the "number of runs" remains a significant issue in terms of applicability to cleaning validation and global harmonization for cleaning validation.

7.2.2 Worst-Case Process Conditions

The traditional approach for cleaning validation protocols has been to include worst-case process conditions in the three protocol runs. Worst-case process conditions may include maximum dirty hold time, maximum batches in a campaign, use of different operators for manual cleaning, shortest allowed time for manual cleaning steps, lowest allowed temperature for manual cleaning processes, and worst-case circuits for CIP skid selection. Parameters such as temperature, cleaning agent

concentration, and process step times for *automated* cleaning processes are generally controlled in a narrow range such that challenging the cleaning process at the lower or upper end of the specification is not appropriate. In this traditional approach, worst-case process conditions may be addressed in each of the three required validation runs, unless there is adequate data from the design and development of the cleaning process to support worst-case conditions in fewer runs.

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8.0 Maintenance of Validated State

A key part of the validation life cycle for any system is maintenance of the validated state. This section deals with activities after the cleaning process has been designed and developed and after the formal validation protocols have been successfully executed. This is critical for cleaning validation, because a lapse in the validated state has the potential to adversely impact the quality, safety and purity of subsequent batches of the same or different products. The main tools for ensuring the continued maintenance of the validated state are change control, risk-based periodic monitoring and data trending review. Additionally, training and retraining are important areas of control for manual cleaning processes, as they are the primary mechanisms for controlling the cleaning cycle. In each of these three areas, knowledge of the design space (see **Section 3.8**) should be applied.

8.1 Critical Parameter Control

In controlling a validated cleaning process, it is of utmost importance to understand the critical parameters used to control the cleaning process. Typically these include cleaning agent concentration, temperature, flow rate and times for all processing steps. During the design phase, an appropriate level of understanding of the process and its variability should be obtained to design a cleaning process capable of addressing this inherent variability. Once the process is well defined, there are a variety of control strategies that may be used.

One control strategy is to set minimum and/or maximum values for each of the key parameters during a cleaning cycle. In this model, each of the steps of the cycle has a defined range that must be monitored and maintained during each execution of the cleaning cycle, and each parameter does not vary outside that range. This approach has an advantage in that that it is straightforward to implement and control.

8.2 Control by Cycle Feedback

Another control strategy is to use analytical feedback to determine cycle step length. For example, the final rinse for a CIP cycle may be continued until the rinse conductivity indicates adequate completion of the rinsing step. This approach has elements of Process Analytical Technology (PAT) (see **Section 11.8**) to ensure the cleaning cycle is appropriately controlled. In the example given, other control parameters, such as temperature and cleaning agent concentration, are maintained in their appropriate ranges. Furthermore, it must be ensured in the design/development steps that conductivity is adequate to measure process step completion. Based on the initial validation, other analytical results (e.g., TOC) may be deemed more indicative of cycle step completion. However, since the cleaning of biotechnology products is accomplished by highly alkaline and/or acidic cleaning agents, conductivity is usually an appropriate indicator of completion of the rinsing step. If one ensures minimum and maximum values are set for other critical parameters and uses these values in concert with control of the rinse time based on analytical feedback, this approach will yield appropriate control of the cleaning cycle.

8.3 Process Alarms

Another key component of applying design space to cleaning processes is alarming of critical parameters. In an automated CIP cycle, alarms may be based on a variety of parameters, such as temperature of the wash and rinse solutions, conductivity of the recirculating wash solution, pressure at the spray device, flow though various circuits, and conductivity of the final rinse. There are a variety of approaches to cleaning the equipment on which an alarm occurred. In all cases, the cause of the

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alarm should be investigated. One strategy is that on specified alarm conditions, the cleaning cycle may be restarted. For example, if inadequate cleaning agent concentration occurred (as indicated by an alarm on the wash cycle conductivity), the cleaning cycle can be restarted from the beginning after appropriate actions are taken to ensure the alarm does not reoccur. This is a conservative approach and ensures a complete cleaning cycle is performed, but care must be taken that alarms are noted and trended to ensure cycle performance is not trending towards being ineffective and to better correct repetitive problems. Alternately, the step in which the alarm occurs may be restarted. This approach strikes a balance between ensuring cycle performance and minimizing cleaning time, as the entire cycle does not have to be repeated. Automated alarming is generally not done in manual cleaning operations. However, if cleaning agent dilution is confirmed by conductivity, or cleaning agent temperature is confirmed by temperature measurement, measurements outside the specified range can serve as an "alarm." In addition, for all cleaning processes, visual inspection after cleaning can serve as an "alarm." In all cases, it must be ensured that cycles performed *during validation* are not "best case" due to alarm conditions. For example, if equipment is soiled, and during the initial validation of the cleaning cycle alarms occur that result in multiple rinse steps being completed, this cycle is no longer representative or worst case, but best case.

8.4 Change Control

A robust change control system is critical to ensuring maintenance of the validated state for cleaning processes. The change control system must cover all key parameters and components of the cleaning system to ensure that all changes with a potential to impact maintenance of the validated state are evaluated. This includes not only changes in the cleaning process, but also changes in equipment and changes in the manufacturing process (for example, a change in temperature in a manufacturing process) which might affect the performance of the validated cleaning process. Quality preapproval and robust tracking of changes are key requirements for this system.

The change control system should provide for a review of each change by an interdisciplinary team. This must include a review of current validation for the equipment being changed, and depending on the nature of the change, may result in laboratory, pilot scale and/or commercial scale evaluations. Significantly major changes may result in the decision that the new cleaning process requires separate validation as a new process. There are some important considerations for designing the test plan to verify changes; review of the design space will assist in this evaluation. First, control parameters must stay within their validated ranges or must be revalidated. For example, if the pump on a CIP skid is validated to deliver water between 5 and 10 liters per minute, and the desired change is to increase the flow rate to 12 liters per minute, new validation testing is required to verify that the pump is capable of delivering the desired flow before validation of the cleaning cycle can occur. Second, the acceptance criteria for analytical methods should remain unchanged from the previous validation unless there is a justified reason for the difference. This is to ensure that changes result in maintenance of the validated state rather than creation of a new state, which may require significant testing to ensure it is still validated. Finally, reduced sample sites and/or fewer analytical methods may be appropriate in many cases to confirm validation based on a change. For example, if the effect of the change is only on bioburden, then it may be appropriate to evaluate only bioburden in studies that evaluate the effects of the change. These differences must be justified in the testing plan/protocol.

8.5 Evaluation of Cumulative Changes

Equally important as a review of each individual change is the review of the *cumulative* impact of changes on a system. This review must provide evidence that the cleaning cycle meets prescribed

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requirements. It is possible that many minor changes (each deemed to have no impact on the validated state) could have an impact when considered in total. This review of cumulative changes should take two approaches. First, a documented analysis (i.e., review of the changes and the impact these changes will have on other parts of the process) of the changes should be undertaken on a regular basis. Second, process performance and alarms must be monitored (as discussed above) to ensure continued maintenance of the validated state and system performance.

8.6 Periodic Monitoring

Another tool for ensuring maintenance of the validated state is a risk-based periodic monitoring program. A periodic monitoring program may provide analytical data to be trended. In most cases involving automated processes, the data are provided by the CIP equipment itself. For example, data may be generated by the CIP skid on wash solution conductivity, final rinse conductivity, temperatures, times and pressure. In other cases, separate sampling may be established for data collection, such as rinse bioburden or TOC. Visual examination after each cleaning process is another type of periodic monitoring. For routine use, however, visual inspection typically does not involve disassembly of equipment solely for the purpose of that inspection.

A documented risk-based approach should be used to optimize compliance in an efficient manner. This could include leveraging family approaches, reduced sample sites and reduced analytical methods. When defining these approaches, the inherent risk associated with a given cleaning process and historical experience/data should be considered. For example, when performing the initial validation on a bioreactor, TOC may be measured via a variety of swab and rinse samples. However, with the proper data analysis, it may be appropriate to measure only rinse TOC during periodic monitoring. Historically it was considered acceptable to perform periodic revalidation on cleaning processes in lieu of periodic monitoring. However, this approach yields a much less robust picture of the state of control of the cleaning process.

8.7 Trending

Trending of cleaning cycle performance, analytical data from routine monitoring, and alarms are another recommendation to ensure continued cleaning cycle performance. When trending any of these data sets, procedures must be in place to initiate an investigation when adverse trends are observed, even if ineffective cleaning cycles have not occurred. Trending of cleaning cycle performance data is important for identifying potential cleaning cycle issues before they result in ineffective cleaning cycles. For example, a slowly increasing trend in the final rinse conductivity may not be indicative of an *ineffective* cleaning process. However, such a trend should require an investigation of the cause. In the example given, it may be that the spray device is becoming clogged, in which case it should be cleaned, and appropriate steps should be taken to prevent clogging in the future. On the other hand, it may be a result of a fouled conductivity sensor. Alarm monitoring and trending will indicate cycle failure, though it will not proactively identify potential issues, as is desired. The incidents of all alarms should still be trended to determine if additional process controls are required to reduce the frequency of alarming.

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9.0 Master Planning for Cleaning Validation

All validation activities should be planned. The requirements for a cleaning validation program should be defined and documented in a master plan or an equivalent document. While in principle the parts of a cleaning master plan may be the same for all drug manufacturing, certain specifics of the master plan for biopharmaceutical manufacturing will be different because of the significant differences between manufacturing and cleaning for large molecule biopharmaceuticals and for small molecules. The plan should provide a description of responsibilities and activities for the planning and execution of cleaning validation. This is best accomplished by a specific cleaning validation master plan. This plan would be described in the overall site validation master plan. The cleaning master plan may be all-encompassing. However, an alternate approach is to have a high-level cleaning master plan and then a cleaning execution or project plan, which has more detailed explanations of the validation requirements. These documents are living documents that should be reviewed and updated on a regular basis. A report to the plan should be written periodically to summarize the major activities executed under the plan during that interval.

The cleaning master plan will describe the overall plan, rationale and methodology to be used in performing cleaning validation. The plan should provide a high level description of the cleaning validation philosophy and strategy that will support the validation activities performed at the site. Detailed procedures on the execution of cleaning validation will be in individual protocols. The plan will define the efforts required to ensure the cleaning program complies with CGMPs. The validation activities are documented according to the requirements of the plan to provide sufficient scientific rationale to assess the suitability of the cleaning program in order to consistently clean equipment to the required specifications. During a regulatory inspection, an inspector may ask to review the master plan and then look at the specific validation protocols and final reports to determine if the plan is appropriate and to assure that the elements of both the plan and individual protocols are being followed.

9.1 Elements of a Comprehensive Plan

The master plan should address each important aspect of the cleaning validation program. Elements of a master plan and the appropriate detail provided for those elements will depend on the practices of the specific facility. Some companies may include more detail in the master plan, while other companies prefer to include that detail in procedures consistent with the master plan. Elements of a master plan may include, but are not limited to, the following topics:

- Purpose of the plan
- Scope of the cleaning program
- Designation of responsibilities
- List of equipment to be validated
- Definitions and glossary of terms
- Prerequisites to cleaning validation (e.g., equipment and utility qualifications)
- Spray device coverage testing
- Use of various cleaning systems (e.g., CIP, COP, mechanical washers or manual cleaning)
- Cleaning reagents and mechanisms
- Cleaning cycle development requirements
- Definition of the production cleaning cycle
- Precleaning methods (e.g., presoaking or inactivation of biologics)
- Soiling solutions
- Definition and use of "worst-case conditions" associated with a cleaning process (e.g., flow rates or step durations)

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- Description of family approach and grouping of products/equipment/systems based on similarities, including an approach to determine "worst-case product" based upon attributes that impact cleaning (e.g., solubility of all components in the "soil")
- Use of dedicated or shared equipment; single use (disposable) equipment
- Definition of circumstances in which cleaning verification is preferred or acceptable (e.g., clinical stages)
- Specific approaches for cleaning upstream vs. downstream bulk process equipment
- Elucidation of approaches for cleaning bulk vs. formulation/fill manufacturing equipment
- Strategies for non-product surfaces, such as lyophilizers
- Use of quality risk management to determine the scope and extent of validation activities
- Establishment of design space based on cleaning parameters and use in ongoing monitoring
- Chromatography and ultrafiltration system requirements
- Use of mock (blank) runs
- Equipment hold study approaches (e.g., dirty hold, clean hold or storage hold)
- Microbial contamination (e.g., bioburden and endotoxin)
- Sampling techniques (e.g., visual inspection, rinse sampling or surface sampling)
- Training/qualification for sampling techniques
- Analytical methods (e.g., validation and recovery requirements)
- Rationale for the use of product-specific assays and nonspecific assays
- Calculations and/or rationales for limits for process residues, microbial contaminants and cleaning agents
- Routine monitoring/validation maintenance
- Change control and revalidation requirements
- References
- Attachments/appendices (e.g., various tables or lists of items within the realm of the plan such as a responsibility matrix or a list of cleaning circuits)

9.2 Harmonization of Site Cleaning Programs

For a product made at more than one site, the cleaning requirements should preferably be the same, where appropriate. For example, if the process equipment scale is different, or the type of cleaning equipment available and/or cleaning process is different (e.g., CIP skid vs. manual), the programs can only be harmonized to a limited degree. The analytical methods used to determine the level of cleanliness should be the same, but the acceptance criteria may differ for any limit that is based on batch size and equipment surface area. The same would also apply to some degree if a contract manufacturer were making the same product. However, there is an additional consideration, since the contractor is also obliged to follow his own master plan. A contract manufacturer may validate their cleaning process using techniques and procedures that differ, but the resulting validation must be compliant and must meet appropriate regulatory expectations. Any critical differences should be addressed up front in the quality agreement with the contractor.

9.3 Cleaning Validation Activities as a Function of Clinical Stage

Validation requirements will vary according to the stage of the product. It may not be feasible to do cleaning validation for equipment processing clinical materials, since typically a limited number of lots are being made, and the manufacturing process may not be locked in yet. Therefore, extensive cleaning process design and development required for cleaning validation is not warranted. In these situations, cleaning verification should be done using testing that is equivalent to that used in the validation program. The analytical methods might not be validated to the same extent as for an

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analytical method used for a cleaning validation protocol. In these cases, their suitability has to be assessed. The test results are used to release the equipment before the next use.

In processing clinical materials, cleaning validation may be possible for process support equipment like buffer and media vessels. The support equipment can be qualified using a worst-case soiling solution in a grouping strategy (see **Section 11.1**). However, in the future, if a new worst-case soiling solution is identified, validation would need to be performed on that new worst case.

Cleaning validation may be considered for late-stage clinicals and is required for commercial manufacturing. It may be acceptable to do cleaning verification for late-stage clinicals, for example, if sufficient lots are not manufactured and cleaned at the same site using the same conditions.

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10.0 Risk Assessment and Management

10.1 Introduction

Quality Risk Management (QRM) is a readily applied and logical process that is effectively used to support the planning and strategy for maintaining a system or a process under continuous quality oversight. The many benefits of a quality risk management process include, but are not limited to:

- Improved planning and preparation to prevent potential failures
- Increased understanding of the critical aspects of systems, processes and products
- Improved stakeholder relationships through better communication
- Increased levels of assurance through documentation of the decision-making process
- Reduced risk to patients by modifying processes to eliminate or reduce risk
- Improved detectability of fault conditions
- Optimization and prioritization of qualification efforts and resources
- Selection of test methods and acceptance criteria which are aligned with critical quality attributes of products
- Compliance with regulatory requirements or expectations
- Assistance in maintaining processes in a state of control

The role of risk management is integral to the design and validation strategy for manufacturing systems. Risk management is a continuous process. Key inputs and data are analyzed and evaluated, and risk mitigation measures are implemented to ensure the outputs of the design are appropriately considered and verified, and that the subject system is demonstrated as fit for purpose. Cleaning and cleaning validation requirements are determined from inputs related to the knowledge of process systems, soils and equipment cleaning aids (e.g., chemical and mechanical features). These requirements are subject to a design review and then verified in accordance with the acceptance criteria that are used to prove that the system requirements have been achieved.

Product knowledge, process knowledge, regulations and quality attributes are used to develop the requirements for cleaning and to define the technologies that will best support the cleaning of manufacturing systems and components. Issues that may impact cleaning include: soil type, cleaning process, equipment design and configuration and availability of utility services. Process knowledge is used to determine CPPs and define CQAs. Examples of each are presented in **Table 10.1** below.

Table 10.1 CPP and CQA Considerations that have Potential Risk Impact to a Cleaning Process

Critical Process Parameters	Critical Quality Attributes
• Process temperature • Process pressure • Process flow • Process time • Cleaning agent concentration • Dirty hold time (soil condition) • Clean hold conditions	• Visual detection or limits • Cleaning agent residues • Product residues • Microbiological residue limits • Drainability/drying • Conductivity/resistivity

QRM involves elements of risk assessment, risk control and periodic review to ensure continuous and effective control. The quality risk management process is best supported by a team of Subject Matter Experts that have an appropriate level of experience from various areas such as operations, technical services, engineering, quality control, quality assurance and regulatory. The experience and diversity of the team provides the opportunity to identify and address all conditions that impact CPP and CQA for the cleaning or manufacturing process.

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10.2 Techniques and Tools for Risk Management and Assessment

Techniques and tools for risk management include process mapping, brainstorming, Hazard Analysis and HACCP, FTA, Cause and Effect Analysis, HAZOP, and FMEA. Risk assessment is initiated early in the life cycle process starting in the planning, development and specification phases of the cleaning process. Risk evaluations are performed periodically. Feedback data is used to make decisions that impact the cleaning process.

Risk analysis is integral to the change management process. The impact of a proposed change is evaluated for quality and safety impacts, and the outcome of the assessment is used to drive the activities that are required to effectively implement the change. Low-risk change tasks (such as an increase in rinsing time) may require little to no additional testing. High-risk change tasks (such as a change in the nature of the cleaning solution) may require a significant level of testing. Risk analysis can also be used to determine the economic impact of a change. It may become evident that a proposed change offers no economic benefit; consequently the change is not implemented.

In summary, quality risk management is a systematic process that involves elements of assessments, development of controls and continuous review throughout the life cycle of the cleaning process. The risk assessment process is effective at identifying CPPs and CQAs. Risk management tools are used to generate data and drive decisions. This information is used to affect risk mitigation, and it reduces risk to an acceptable level. Risk assessments should be documented so that critical factors are identified, decision pathways are understood, and the information is effectively communicated to the stakeholders.

11.0 Special Considerations

11.1 Grouping/Family Approach

Grouping is a strategy whereby similar manufactured products and/or equipment are considered together, and a formal protocol is performed on a representative from the group. The representative from the group is usually the worst case among products or equipment in a group. Grouping is also called matrixing, family approach and bracketing. The rationale for grouping is to eliminate non-essential or non-value-added work based on a risk approach. One requirement for grouping is that product and equipment be cleaned by the same cleaning process.

11.1.1 Product Grouping

Products are assessed for their relative cleanability, typically in laboratory studies. Laboratory evaluations discussed in **Section 3.0** may be utilized. Cleanability is assessed using representative surfaces, with stainless steel being the most common because of its predominance in biotechnology equipment. From the results, the relative cleanability of each product is defined, typically by determining under proposed cleaning parameters which product requires the longest time to clean. A Performance Qualification (PQ) protocol, usually involving a minimum of three validation runs because this protocol represents other products, is performed. The acceptance criterion for that worst-case product is generally the most stringent acceptance criterion of all products in the group. Since it is common in biotechnology to have the same acceptance criteria and same analytical methods for similar products (bulk actives, finished drug products, intermediates, etc.), selection of the acceptance criteria is usually straightforward. Successful cleaning validation of the representative (worst- case) product means the cleaning of the other products in the group is also validated.

11.1.2 Equipment Grouping

Grouping of equipment is an effective method for encompassing equipment from a limited population of systems undergoing cleaning validation without redundant testing. The grouping strategy is based on designating equipment as "identical" or "similar," based on design and cleanability. Once equipment has been placed within a designation, the designation defines the cleaning validation requirements. If it involves *identical* equipment, a protocol with a minimum of three validation runs involving any combination of three equipment items in the group is performed. Provided an adequate rationale is given for determining the equipment items are identical, there is no need to perform validation runs on every item in the group. For *similar* equipment, the representative equipment is the worst case or may involve bracketing of equipment. For example, for storage tanks of the same size but of different complexity, such as the number of baffles, the more complex equipment is chosen as the worst case. For similar equipment of different sizes, the largest and smallest (representing the extremes) may be chosen for the formal validation runs (unless one size can be determined as the worst case). Confirmatory validation runs (perhaps only one run) are an option for other equipment (not a worst case) within the group.

11.1.3 Introduction of a New Product or New Equipment Into a Group

The introduction of a new product into an already validated group is then assessed using the same evaluation process to initially determine the worst-case product. It is recommended that when each new product is tested, a suitable control, such as the previous worst-case product, is included. Relative product cleanability is then used to determine validation requirements for that product on equipment used for other products in that group. The relative cleanability of the product in relation to the preceding worst-case product will dictate the validation requirements. Based on a risk assessment,

introduction of an *easier-to-clean* product may just require laboratory and/or scale-up studies to confirm ease of cleaning or may require a confirmatory validation protocol. Introduction of a *more difficult-to-clean* product will require validation of that new worst-case product.

Based on risk considerations, introduction of new *identical* equipment may just involve determination that it is equivalent or may require an additional confirmatory validation protocol. Introduction of new *similar* equipment requires an evaluation if that new equipment represents a new worst case or a new extreme. If not a new worst case or new extreme, a confirmatory validation protocol using only a visually clean criterion can be used. If the new equipment is a new worst case or extreme, the validation requirements for the previous worst case or extreme should be repeated for the new worst case or extreme equipment.

11.1.4 Conclusion

The use of product and equipment grouping may be used to streamline cleaning validation programs while ensuring sufficient data to support the validation of procedures, processes and equipment associated with cleaning. The grouping program for a given facility or company should be in a well-defined validation program/validation master plan.

11.2 Cleaning Agent Issues

Equipment cleaning processes in the biopharmaceutical industry often involve a pre-rinse with water, an alkaline wash, an acid wash and a series of water rinses.

11.2.1 Sodium Hydroxide Wash

A commodity alkali such as sodium hydroxide is often used for the alkaline wash step. The high pH and alkalinity of sodium hydroxide solutions enhance solubility of most organic process residues, and in some cases facilitate hydrolysis. Sodium hydroxide is also widely available, relatively inexpensive and, being a single component, relatively easy to analyze and validate for cleaning agent removal. Commodity cleaners such as sodium hydroxide, however, may have limited effectiveness for tenaciously adhered or baked-on proteinaceous residues, cell debris and antifoams. They also have limited wetting characteristics and soil suspending ability. The higher pH of sodium hydroxide also facilitates the precipitation of salts or oxides of such ions as calcium, magnesium and iron, if those ions are present during the cleaning process.

11.2.2 Acid Wash

The addition of an acid wash step after the caustic wash may overcome precipitation and buildup of inorganic compounds and help broaden the spectrum of soils cleaned although at the expense of adding another cycle. In addition, maintaining a clean surface and limiting the deposition and buildup of iron or other anodic contaminants may help minimize the potential for stainless steel corrosion and rouge formation.

11.2.3 Formulated Detergents

Formulated detergents are multi-component cleaning agents that take advantage of several different cleaning mechanisms, thus providing broader spectrum effectiveness. In addition to the mechanisms of alkalinity and hydrolysis offered by a commodity caustic, a formulated alkaline detergent might

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provide improved wetting and soil penetration, emulsification, chelation of calcium, iron or other inorganic ions, and might facilitate dispersion of particulates in one wash step. Despite the use of chelating agents and the broad spectrum effectiveness of formulated detergents, rouge buildup may still be observed over a period of time, and a periodic derouging process may be necessary, particularly in applications that involve aggressive process conditions such as SIP.

11.2.4 Issues in Selection

A number of factors besides broad spectrum cleaning effectiveness need to be considered when selecting detergents. These include rinsability, quality, consistency, substrate compatibility, stability, safety, toxicity, assay suitability, environmental compliance and assured long-term availability.

11.3 Special Equipment Issues

11.3.1 Chromatography Columns

Chromatography columns are typically used in protein purification processes. In contrast to equipment like fermenter vessels or tanks used in purification that are cleaned empty, for batch-to-batch cleaning within a campaign of the same product, the columns are clean packed with resin after the batch is processed. The cleaning processes for the chromatography resin packed into the column are process specific and depend on the type of resin used. Resin cleaning and reuse is out of scope of this document and is described in detail in PDA *Technical Report No. 14, Validation of Column-Based Chromatography Processes for the Purification of Proteins.* ***(19)***

Since chromatography columns are cleaned with resin packed into a column, the resin cleaning process also cleans the column housing. Therefore, after unpacking a column, product- and process-related impurities on the column surfaces are already removed to a certain extent. However, it is common practice to clean an empty column after a column is unpacked. Column frits or sieves are normally product dedicated due to their porosity and the difficulties to validate removal of product- and process-related impurities. In moving from a campaign of one product to a new campaign of a different product, they are removed prior to column cleaning and stored for further use. Typically, a manual cleaning process using the same or similar cleaning agents as for tank cleaning is used for the cleaning of the column housing. After cleaning, the same cleaning validation principles (such as limits) applied to tanks can be utilized.

11.3.2 Tangential Flow Filtration (TFF) Filter Systems

Similar to chromatography columns, tangential flow filtration filter housings (also called filter holders) are cleaned together with the membranes after a batch has been processed. The cleaning processes for the filter membranes packed into the filter housing are process specific and depend on the type of membrane used. TFF membrane cleaning is out of scope of this document and is described in detail in PDA *Technical Report No. 15, Validation of Tangential Flow Filtration in Biopharmaceutical Applications.* ***(20)***

Since TFF membranes are cleaned in a filter housing, the membrane cleaning process also cleans the filter housing. Therefore, after removing TFF membranes, product and process related impurities on the housing surface are already removed to a certain extent. However, it is common practice to clean an empty filter housing after the membrane cartridge or cassette is removed. Typically, a manual cleaning process using the same or similar cleaning agents as for tank cleaning is applied for the cleaning of the TFF housing. After cleaning, the same cleaning validation principles (such as limits)

applied to tanks can be utilized.

11.3.3 Centrifuges

In many biotechnology processes, centrifuges are used at the end of fermentation to remove cells from cell cultures or to separate bacteria from the fermentation broth prior to further processing. Many centrifuges can be cleaned in place; others have to be manually cleaned.

Cleaning complex pieces of equipment like centrifuges can be challenging. For instance, not all surfaces which have been in contact with the fermentation broth can be easily reached. Special attention has to be given to hard-to-access areas of the equipment, both in the cleaning process and in the evaluation of that cleaning. After cleaning, the same cleaning validation principles (such as limits) applied to tanks can be utilized.

11.4 Multi-Host Facilities

Cleaning validation is performed to demonstrate that residual material or cleaning agents remaining on shared equipment surfaces following the manufacture of one product are controlled to below acceptable levels so that the shared equipment may be utilized for the manufacture of a subsequent product without impacting the safety, identity, strength, quality or purity characteristics of the subsequent product. In order to maintain a successful cleaning validation program for a multi-host facility involving both cell culture and bacterial fermentation processes, the validation strategy must consider not only cleaning agent and process residues, but also specific requirements for each process step necessary to maintain drug quality throughout the manufacturing process.

Robust cleaning validation programs for multi-host facilities should ensure that cleaning procedures are appropriate for all processes/systems used in the facilities. The successful cleaning validation program for a multi-host facility will ensure the cleaning/sanitization/changeover procedures control residual process residues to below acceptable levels for all products made in the facility.

11.5 Non-product Contact Surfaces

Non-product contact surfaces may be defined in different ways by manufacturers. One way is to regard any equipment surface that does not directly contact the drug substance (the active) or drug product as non-product contact. Examples under this definition might be lyophilizers, equipment used solely to manufacture and transfer buffers and media, and equipment to process drug product after completion of primary packaging. Other companies may choose to define some of these surfaces as "indirect product contact," since in the case of buffers and media, any residues left on equipment surfaces after cleaning will contact the next buffer/media and will eventually contact the drug substance manufactured with that next buffer/media. Because of the limited impact of these indirect or non-product contact surfaces, requirements for cleaning validation can be reduced or cleaning validation can be eliminated in certain situations.

11.5.1 Equipment for Buffers

For buffers, which generally have components that are readily water soluble, cleaning is generally relatively easy and may be done with either water alone or a dilute caustic solution. Concerns about cross-contamination of buffers are not necessarily based solely on the carryover of the buffer components, but on any effects residues might have on production efficiency or production quality.

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Based on a risk analysis, cleaning validation of buffers may only involve acceptance criteria of visually clean and conductivity. Although some companies may also choose to include a measurement of TOC, conductivity is the better method because of the fact that the buffers are readily water soluble and highly conductive. Measurement of bioburden may also be utilized, depending on a risk analysis based on the growth promotion properties of the buffer. Measurement of specific residues may be appropriately done by rinse sampling because of the water solubility of the buffer components. Grouping of buffers and selecting the worst case for cleaning validation is also a valid (and common) approach for cleaning validation of buffers.

11.5.2 Equipment for Media

The situation with media is similar to that of buffers, except that media are generally much more difficult to clean, such that cleaning solutions containing alkali are used. Concerns about media carryover are also not necessarily based on safety concerns related to carryover of the media components, but on any effects that media residues might have on production efficiency or production quality of drug substance made utilizing the next media batch. Cleaning validation may include the criteria of visually clean, with measurements of TOC, conductivity and/or bioburden after cleaning. TOC is used because of the organic nature of the media components. Conductivity confirms removal of the alkaline cleaning solution. Bioburden is measured because the media typically enhances microbial growth. For concerns about endotoxin from gram-negative bacteria, endotoxin may also be evaluated. Because of the design of equipment for media manufacture, rinse sampling alone may be adequate. However, the solubility or degradation of media components should be considered as part of the risk analysis for performing rinse sampling only. Grouping of media and selecting the worst case for cleaning validation is also a valid (and common) approach for the cleaning validation of media.

11.5.3 Lyophilizers

Acceptable (meaning saleable and meeting all product specifications) drug product never touches lyophilizer surfaces; contact of drug product with lyophilizer surfaces generally only occurs because of broken vials or vials that tip over and spill during loading. However, because of the close proximity of lyophilizer shelves to open product, and because of a perceived airborne transfer of residues on the shelves to open vials, cleaning validation is typically performed on lyophilizers used in formulation/fill operations. Typically, only WFI is used for cleaning lyophilizers because of the concern of leaving cleaning solution residues inside the lyophilizer. Cleaning procedures for lyophilizers may also include a precleaning step to remove broken glass or spilled product before the validated cleaning procedure is performed. Carryover calculations for setting limits are typically not applicable to lyophilizers because the *indirect* contact with the next product precludes any scientifically based calculation as conventionally performed for *direct* product contact surfaces. The most common acceptance criteria for cleaning validation of lyophilizers are a visually clean requirement and/or a measure of TOC. Since direct carryover calculations are not applicable, TOC limits are typically based on one of the following criteria: a 10 ppm TOC criterion in any desorbed swab sample, or a TOC limit the same as the TOC limit for any *direct* product contact equipment immediately before or after the lyophilizer. The logic of the latter approach is that indirect contact equipment is *less* of a risk than direct product contact equipment; therefore, if the indirect is held to the *same* requirement as the direct, then the product should be acceptably protected. Bioburden may also be measured during cleaning validation of lyophilizers; however, lyophilizers generally undergo an SIP process after cleaning.

11.5.4 Packaging Equipment

Once the drug substance is in its primary packaging, the risk of cross-contamination is relatively low. Cleaning processes should be used on the packaging lines after primary packaging but do not require cleaning validation. The main concern with cross-contamination is broken vials, which release product. Cleaning processes for such situations should be considered; however, because contamination of the next product may only involve contamination of the outside of the primary packaging, cleaning validation becomes a major concern only if that spilled product has some unusual toxicity concerns. In those cases, a dedicated line or a cleaning step known to deactivate or degrade that drug active should be considered. Such a degradation process may appropriately be confirmed in a laboratory study demonstrating degradation or deactivation of the active.

11.6 Viruses, Mycoplasma and Prions

The biological nature of materials and processes used in biotechnology production presents unique challenges for equipment cleaning and cleaning verification. Besides product and product-related residues and those residues that may remain from the cleaning process itself, viruses, mycoplasma and prions are another concern for product contamination. However, viruses are not routinely addressed in cleaning validation protocols or programs, but in viral clearance studies and/or as part of process validation.

11.6.1 Control Steps

While the primary control measures are viral clearance process steps, testing for viruses and mycoplasma in the unprocessed bulk, and exclusion of raw materials that might contain viruses, mycoplasma and prions, specially designed cleaning processes might also be needed in some cases. Control of raw materials is essential, especially for plasma and plasma-derived products and those derived from animal materials – through vendor certification, incoming QA inspection and QC testing. Recombinant and "non-animal origin" materials should be used wherever practicable throughout all processing steps; however, viruses have been found to contaminate non-animal raw materials due to exposure during raw material storage, either at the raw material vendor or at the manufacturing site due to adventitious viral contamination. Also, mycoplasma that can replicate in mammalian cell culture often have a plant source and may be a contaminant in plant peptones. Since mycoplasma contamination can also be due to humans, proper gowning and personal hygiene are critical to control contamination. Additionally, sterilization of equipment used in cell culture, fermentation and finished product manufacturing may provide additional assurance of product that is free of viruses and mycoplasma.

11.6.2 Control by Cleaning

Equipment cleaning using caustics and/or acids at appropriate ranges for the cleaning parameters of time, temperature, concentration and action is also essential to successful biocontamination control. The use of cleaning solutions containing sodium or potassium hydroxide is widely practiced in the industry. NaOH has been shown to be effective for inactivating most viruses. A U.S. FDA/CBER guidance provides a regulatory perspective on prion inactivation methods:

"TSE agents are quite resistant to most disinfecting regimens. There is no current consensus on specific details of decontamination requirements for blood products. However, methods of destruction of TSE-implicated material include steam autoclaving at 132°C for 1-4 hours, incineration, or treatment

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with 1 N NaOH or concentrated sodium hypochlorite for at least 1 hour. These treatments are known to diminish (but may not completely eliminate) infectivity." ***(21)***

11.6.3 Conclusion

Although cleaning processes are generally not designed to remove viral, mycoplasma or prion contaminants, a well-designed, robust cleaning procedure can be an effective process partner in a facility's overall biocontamination control strategy.

11.7 Single-Use Equipment

Single-use equipment or components may be considered in place of reusable equipment that requires cleaning. Single-use technology has significantly evolved over the last decade and is being rapidly implemented in biopharmaceutical manufacturing since the introduction of the single-use bioreactor. Single-use equipment may include anything from carboys, storage bags, bioprocess containers, filter systems, tubing and connection devices to bioreactors. Many of these components are available presterilized (e.g., by gamma irradiation). Such single-use items offer possibilities to simplify the handling of critical process steps and significantly reduce contamination risks, especially for multiproduct facilities and for contract manufacturers. The economic and operational advantages of single-use equipment stem largely from eliminating cleaning and sterilization, reducing the utilities that support these operations, and enabling rapid equipment setup and turnaround.

Along with the benefits of single-use equipments, there are risks and limitations to consider. Most single-use items are polymeric materials. All polymeric product-contact materials and components used in cGMP manufacturing must be assessed to determine if the polymer is safe, and if it is compatible with the solution it is in contact with. Thorough evaluation of potential extractables and leachables is necessary to ensure the safety and quality of the drug product and to maintain compliance with appropriate regulatory requirements for extractables/leachables. Most companies address this as part of process validation and/or qualification of the single-use item.

11.8 Process Analytical Technology

PAT is defined by the U.S. FDA to be "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality." ***(22)*** The U.S. FDA further notes that "the term 'analytical' in PAT is viewed broadly to include chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner." Much has been published about PAT in general and about PAT in many processes; the reader should consult those references for general background on PAT. However, there are limited publications about PAT in cleaning processes and cleaning validation ***(23-26)*** as compared to PAT for other manufacturing operations. The emphasis for PAT here is for the use of a feedback loop from the analytical measurement to control a cleaning process or cleaning process step. It should be noted that consistent with PAT principles, the timely measurement could be in-line, on-line or at-line.

"Timely measurements" have long been used in cleaning processes to assist in the design of *rinse cycle times* in automated CIP systems, including those in the biotechnology industry. For example, a common practice in the design of the rinsing process has been to measure conductivity of the final rinse as a function of rinse time. Conductivity is a useful parameter for this determination, since cleaning in biotechnology manufacturing usually involves highly alkaline and/or acidic cleaning

agents, which possess significant conductivity (in addition to the conductivity of the manufactured product and/or its degradants). If evaluated over several cleaning process runs in the design phase, a minimum time to consistently complete the rinsing process can be effectively determined. A safety factor (additional time) may be included as part of this determination. While such a study in the design phase would be appropriate for a PAT application, unless it combines the timely measurement with a feedback mechanism to control the cleaning process during commercial cleaning processes, it would not be considered PAT. As described *in this paragraph*, the purpose of the timely measurement is not to control the rinsing process, but to assist in selecting a *fixed* rinse time.

11.8.1 PAT for Cleaning Process Control

The more relevant use of PAT for cleaning processes is the use of a timely measurement to define the completion of a cleaning process step. In this case, the achievement of a certain analytical measurement is a controlling mechanism for completion of that step. In the situation referred to previously about measuring conductivity online, if it is possible to determine that the achievement of a certain conductivity correlates with the end of the rinsing process, conductivity could be employed in a PAT approach. That is, the rinse time is not fixed, but could be variable depending on the time needed to achieve that predetermined conductivity value. In addition, consistent with PAT principles, it would be expected that the achievement of that conductivity value would be within a defined time window. The U.S. FDA PAT guidance states "Within the PAT framework, a process end point is not a fixed time; rather it is the achievement of the desired material attributes. This, however, does not mean that process time is not considered. A range of acceptable process times (process window) is likely to be achieved during the manufacturing phase and should be evaluated, and considerations for addressing significant deviations from acceptable process times should be developed." ***(22)*** For example, achievement of a desired conductivity in a very short time could be due to insufficient cleaning solution in the cleaning process. Achievement of the desired conductivity in a very long time may be the result of a clogged spray device. In both cases, a final conductivity is recorded and a final rinse time is recorded. However, in the traditional approach time is the step-controlling parameter, and conductivity is the monitoring parameter. In a PAT approach, conductivity could be the step-controlling parameter, and time would be the monitoring parameter.

Sometimes there is an inappropriate objection to the use of PAT in this way, because it seems to violate the cleaning validation principle of not cleaning until clean (or testing until it's clean). However, one of the features of PAT is that traditional rules of what is done for validation may not apply. As noted in the U.S. FDA's PAT guidance, "Systems that promote greater product and process understanding can provide a high assurance of quality on every batch and provide alternative, effective mechanisms to demonstrate validation (per 21 CFR 211.100(a), i.e., production and process controls are designed to ensure quality). In a PAT framework, validation can be demonstrated through continuous quality control whereby the process is continually monitored, evaluated, and adjusted using validated in-process measurements, tests, controls, and process end points." ***(22)***

While this example of conductivity as a timely measurement to control the rinse process has been used, there are at least *theoretically* other opportunities for timely measurement to assist in the cleaning process design. For example, timely TOC measurements during the washing step may be indicative of the minimum time needed to complete the washing step (before rinsing is initiated). By this, it is meant that as proteinaceous soils are removed from the equipment surfaces in the washing step, it would be expected that the TOC in the wash solution would increase and then level off at a time when no more soil is removed (that is, the wash step is complete).

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11.8.2 PAT Measurement Tools for Biotechnology Cleaning Processes

Currently, the most common tools with potential PAT application in biotechnology cleaning processes are conductivity and TOC, because these can be measured online in the cleaning or rinse solution. Surfaces techniques, such as NIR for surfaces, may not be practical for *timely* control, because such techniques involve measuring for residues *after* the cleaning process is completed, not during the cleaning process.

Conductivity sensors are readily available for in-line measurements and have been widely used for in-line monitoring (but not necessarily for control). Online TOC does not involve an in-line sensor, but rather a "sipper tube" which diverts a stream from the process piping to the online instrument (U.S. FDA calls this "on-line in a diverted stream"). One concern about the use of TOC in this way is the delay between taking the sample and the output of the actual measurement. Another concern is that if the instrument is continually taking and measuring samples during a cleaning process, earlier samples with high TOC values may carry over to the following sample and cause a false high reading. Of course, if the process is performed until the desired TOC value is achieved, there is an assurance that the process is adequate, because that possible carryover situation reflects a worst case.

11.8.3 Additional Considerations for PAT

It should be noted that in the conductivity example described in **Section 11.8.1**, all aspects of traditional cleaning validation are not avoided. If conductivity were the measure of a residual cleaning agent, and if only sampling rinse water were acceptable for determining residues of a cleaning agent, a PAT approach of measuring conductivity as a rinse step control parameter would also provide assurance that the cleaning agent was adequately removed for each and every cleaning process. However, it would not address issues of residues of the active and/or bioburden. Those residues would have to be measured in the traditional manner, unless a timely measurement of those residues could be utilized.

It should be clarified that rapid and/or online methods by themselves do not necessarily constitute PAT. As discussed previously, online conductivity can be a routine monitoring tool in a cleaning process step without controlling a process step. Online TOC (other than during the design phase) is not the use of PAT, unless the achievement of a certain analytical measurement of TOC determines and/or controls the completion of a cleaning process step. The same is the case with rapid microbiological methods. Rapid methods may enable one to obtain lab data faster, but unless those measurements determine and/or control the end of a process step, they are just rapid monitoring tools, not PAT tools (although they have the potential to be PAT tools).

The examples given illustrate the use of PAT for process design and for process step completion. In an ideal world, PAT would be used for real-time release of cleaned equipment and would be used instead of cleaning validation. However, at this time the tools to utilize PAT to confirm that equipment surfaces are appropriately clean (measuring removal of active, cleaning agent, bioburden and endotoxin in the case of biotechnology manufacturing) have not been adequately developed to enable real-time release for cleaning biotechnology equipment.

11.9 Product Changeover

Much biotechnology manufacturing involves campaigning. In a campaign, the same product is made again and again. However, typically between each batch in a campaign, validated cleaning is performed. At the end of a campaign, some additional steps may be taken to prepare the equipment

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for the subsequent campaign of a *different* product. This extra precaution typically involves performing an additional cycle of the same validated cleaning process used for cleaning between batches in a campaign. Because of concern about possible migration of residues (particularly product active) into gasket materials, or more accurately, into the interstices between gaskets and stainless steel surfaces, changeover of soft parts such as gaskets may also be done after the initial cleaning. During changing of soft parts, a more comprehensive visual examination of the equipment surfaces is made. Following reassembly of the equipment, the validated cleaning process is repeated. Routine monitoring of both the initial cleaning and the final cleaning is performed as is normally done. Some companies also might perform a specific analytical test (such as an ELISA procedure) as an extra check for the previous active protein in the final rinse water of the second cleaning . It should be recognized, however, that the likelihood of any native protein surviving one cleaning process, much less two cleaning processes, is very remote.

An alternative is not to change out those soft parts based on data showing no migration of residues into interstices between gaskets and stainless steel surfaces or analysis based on potential carryover. Such data can be based on studies on commercial equipment, on scale-up equipment, and/or in a laboratory simulation. In such cases, one validated cleaning cycle is used both between batches of one campaign and for a campaign changeover.

Certain equipment is generally dedicated to a given product. This includes chromatography resins and ultrafilters. Cleaning may be done on these items within a campaign; however, at the end of the campaign the resins and ultrafilters are cleaned, but typically are not used for campaigns involving different products.

11.10 Clean Hold Considerations

Following cleaning, equipment that is to be reused should be stored in a manner to protect it from contamination during storage. Criteria used to determine acceptability of storage conditions may include lack of bioburden proliferation, endotoxin level and visual examination. A major regulatory concern is the control of bioburden proliferation during the storage of equipment. Even if equipment is sterilized prior to use, it is prudent to measure bioburden after the clean hold time to ensure that the subsequent sterilization is not excessively challenged. This is also important from the standpoint of the control of pyrogens from gram-negative bacteria, which may not be removed or inactivated by sterilization processes. ***(8)*** Storage instructions should be specified in a control document, such as the cleaning procedure or approved storage procedure.

The best procedures are to store cleaned equipment in a dry state or in a solution that inhibits the proliferation of bioburden. If equipment is to be stored in a dry state, manufacturing controls should be in place to ensure that equipment is sufficiently drained and dried upon completion of the cleaning process, as well as to minimize the amount of condensed water accumulation in the equipment after cleaning due to equipment cooling. In addition, it is preferred that equipment be stored in a manner to prevent external recontamination. If stored in a dry state (that can be unequivocally established as dry), and if protected from external contamination, formal studies to demonstrate lack of bioburden proliferation may not be necessary. Based on sound scientific principles, bioburden will not proliferate on clean, dry surfaces. If stored in an inhibiting solution, the solution should be known to inhibit bioburden growth (such as dilute caustic) or data should be developed to demonstrate inhibition.

If the equipment is stored with a possibility of water in all or parts of the equipment, there are two common strategies to control microbial proliferation during the storage of equipment. One strategy is to establish an acceptable time between the end of cleaning and the beginning of the next use

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(which may be sterilization, sanitization, or a manufacturing process step) by performing a clean hold validation. After a predetermined storage time, sampling by a suitable method is performed and the post-hold data is compared to the data at the beginning of storage. If rinse sampling is used, it should be ambient temperature water so that what is measured is the bioburden remaining on surfaces (the use of a hot water rinse may reduce the bioburden in the rinse solution). Bioburden (and possibly endotoxin) levels in the equipment are measured to ensure that levels would not challenge the sterilization or sanitization procedures or exceed in-process manufacturing specifications.

If clean hold validation is not performed, or if the validated clean hold time is exceeded, a validated water (usually hot water) flush may be used before sterilization, sanitization, or use of the equipment to reduce any microbial proliferation that might have occurred during storage to an acceptable level before further manufacturing or processing on the equipment. After the water flush, sampling (by rinse, swab or plating) is performed. Bioburden (and optionally endotoxin) levels in the equipment are measured to ensure that levels would not challenge the sterilization or sanitization procedures or exceed in-process manufacturing specifications.

For clean hold time studies using rinse water being fed from process lines, a few common approaches to establishing the acceptable amount of rinse water to use are based on the minimum working volume of the system or the minimum CIP rinse based on the design. Bioburden values in any rinse sample should be compared to the measured bioburden values based on the equivalent rinse sampling at the beginning of storage. It is preferable to collect the entire volume of rinse solution and agitate it for a specified period of time to ensure homogeneity before collecting the sub-sample for testing.

For buffer and media vessels, when operational controls are in place to minimize bioburden, and when a risk assessment demonstrates that there is minimal risk to product quality as a result of the control procedures, a clean hold validation may not be necessary.

Validation of clean hold studies on a given piece of equipment should be able to be applicable to all products using that equipment and to all cleaning processes for that equipment, provided the final state of the cleaned equipment and the storage conditions are consistent. If a validated clean hold time is exceeded, an assessment should be made as to the need for corrective action. Appropriate corrective actions before use or further processing may include cleaning the equipment again using a validated cleaning process or using a validated hot water rinse (as described above) to bring bioburden to an acceptable level. If any changes to the equipment, manufacturing processes and/or cleaning procedures are made, the impact of these changes on the clean hold studies should be evaluated.

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12.0 Regulatory Issues

Most regulatory documents dealing with cleaning validation do not make any explicit comments about biotechnology manufacturing or about how cleaning validation might be different for biotechnology as compared to other pharmaceutical manufacturing. The general principles laid out in regulatory documents, i.e., limits should be "practical, achievable, and verifiable," ***(8)*** apply equally to biotechnology manufacturing and small molecule pharmaceutical manufacturing.

Below are specific regulatory comments relevant to biotechnology cleaning validation:

1. The WHO Working document QS/03.055/Rev.1 includes a statement about the use of ELISA as an analytical technique for biopharmaceuticals. ***(27)*** However, that statement is not of much help, since most biopharmaeutcials degrade in the cleaning process.
2. The U.S. FDA's "Q&A on CGMP" (updated 2005) provides a rationale for allowing the use of TOC for cleaning validation purposes. ***(28)*** While it does not specifically mention use for biotechnology, the biotechnology industry is among the biggest users of TOC for cleaning validation.
3. The U.S. FDA inspection guide for biotechnology has the following statements about "cleaning procedures:"

"Validation of the cleaning procedures for the processing of equipment, including columns, should be carried out. This is especially critical for a multi-product facility. The manufacturer should have determined the degree of effectiveness of the cleaning procedure for each BDP [Biotech-derived product] or intermediate used in that particular piece of equipment.

"Validation data should verify that the cleaning process will reduce the specific residues to an acceptable level. However, it may not be possible to remove absolutely every trace of material, even with a reasonable number of cleaning cycles. The permissible residue level, generally expressed in parts per million (ppm), should be justified by the manufacturer. Cleaning should remove endotoxins, bacteria, toxic elements, and contaminating proteins, while not adversely affecting the performance of the column." ***(29)***

Following these two paragraphs are additional comments on cleaning procedure, limit and analytical/sampling issues. However, other than the explicit comment about including residues of bacteria and endotoxin, there is little that is specific to biotechnology manufacturing.

4. The U.S. FDA guidance for lyophilization of parenterals states the following:

"One could conclude that if contamination is found on a chamber surface after lyophilization, then dosage units in the chamber could also be contaminated. It is a good practice as part of the validation of cleaning of the lyophilization chamber to sample the surfaces both before and after cleaning." ***(30)***

This probably means that if contamination of shelves from external sources (such as hydraulic fluid) is found after lyophilization (and before cleaning), it is likely that the same contaminant is in vials. However, if that is of concern, that is a maintenance issue and probably belongs as part of preventive maintenance rather than cleaning validation. Note that this statement has an *implicit* assumption that cleaning validation is performed for vial lyophilization.

5. The Health Canada, Health Products and Food Branch Inspectorate Guidance, *Cleaning Validation Guidelines* ***(11)*** states the following about biotechnology manufacturing:

"Relevant process equipment cleaning validation methods are required for biological drugs because of their inherent characteristics (proteins are sticky by nature), parenteral product purity requirements, the complexity of equipment and the broad spectrum of materials which need to be cleaned."

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It furthermore states the following about bracketing (grouping) for biotechnology manufacturing:

"For biological drugs, including vaccines, bracketing may be considered acceptable for similar products and/or equipment provided appropriate justification, based on sound, scientific rationale is given. Some examples are cleaning of fermentors of the same design but with different vessel capacity used for the same type of recombinant proteins expressed in the same rodent cell line and cultivated in closely related growth media; a multi-antigen vaccine used to represent the individual antigen or other combinations of them when validating the same or similar equipment that is used at stages of formulation (adsorption) and/or holding. Validation of cleaning of fermentors should be done upon individual pathogen basis."

6. ICH Q7 *Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients* ***(10)*** contains in **Section 18.0**, "Specific guidance for APIs manufactured by cell culture/fermentation." It provides GMP guidance on the cell culture/fermentation manufacturing process for biotechnological product and some small molecules.

At the beginning of the section, it states that, "in general, the degree of control for biotechnological processes used to produce proteins and polypeptides is greater than that for classical fermentation processes." It further explains that, "APIs produced by classical fermentation are normally low molecular weight products such as antibiotics, amino acids, vitamins, and carbohydrates."

Q7 states the following regarding equipment cleaning for cell culture/fermentation:

"Cell culture equipment should be cleaned and sterilized after use. As appropriate, fermentation equipment should be cleaned, sanitized, or sterilized."

"Shared (multi-product) equipment may warrant additional testing after cleaning between product campaigns, as appropriate, to minimize the risk of cross-contamination."

Q7 states the following regarding equipment cleaning for harvesting, isolation and purification:

"All equipment should be properly cleaned and, as appropriate, sanitized after use. Multiple successive batching without cleaning can be used if intermediate or API quality is not compromised."

Q7 states the following regarding equipment cleaning for viral removal/inactivation steps:

"The same equipment is not normally used for different purification steps. However, if the same equipment is to be used, the equipment should be appropriately cleaned and sanitized before reuse. Appropriate precautions should be taken to prevent potential virus carryover (e.g., through equipment or environment) from previous steps."

7. ICH Q5A, *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* states the following regarding validation of column cleaning and regenerating from viral inactivation perspective:

"Assurance should be provided that any virus potentially retained by the production system would be adequately destroyed or removed prior to reuse of the system. For example, such evidence may be provided by demonstrating that the cleaning and regeneration procedures do inactivate or remove virus." ***(31)***

While these are specific comments from guidance documents, it should be recognized that regulatory inspectors may (and should) have additional expectations for cleaning validation in biotechnology based on current industry practices and on their past experience with similar companies.

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15.0 APPENDIX – CARRYOVER CALCULATIONS

Note: In the calculation examples that follow, the recovery based on sampling (percent recovery) is not included. For companies that utilize the sampling recovery to "correct" the limit, that factor should be included in their calculations.

Example 1: This example is based on the dose of the *active* for *formulation/fill* manufacturing. It is based on a 1/1000 minimum therapeutic daily dose of the cleaned active.

MinTD:	Minimum Therapeutic Daily Dose of the active of cleaned product
MBS:	Minimum Batch Size of next *drug product* made in the same equipment
MaxDD:	Maximum Daily Dosage of next *drug product* made in the same equipment
SF:	Safety Factor

Example for products A and B: if

- MinTD = 25 mg (or 25,000 µg)
- MBS= Minimum Batch Size of the following *drug product* B = 1000 L
- MaxDD= Maximum Daily Dosage units of *drug product* B = 10 mL
- SF = 1000

The limit in the next product is calculated by dividing the MinTD by the SF and the MaxDD:

$$\text{Limit in next product} = \frac{25{,}000\ \mu g\ (\text{MinTD})}{1{,}000\ (\text{SF})\ X\ 10\ mL\ (\text{MaxDD})} = 2.5\ \mu g/mL$$

Since this calculated value is *more stringent* than 10 ppm (10 µg/g, or approximately 10 µg/mL), this value will be used for subsequent calculations.

The Maximum Allowable Carryover (MAC) is calculated by multiplying the limit in the next product by the MBS:

MAC = 2.5 µg/mL X 1,000,000 mL = 2,500,000 µg

The limit per surface area can then be calculated by dividing the MAC by the shared surface area between the two products. Continuing with the same example, if the shared surface area is 120,000 cm^2, then the limit per surface area is:

Limit per surface area = 2,500,000 µg/120,000 cm^2 = 20.8 µg/cm^2

The limit (mass) per swab can be calculated by multiplying the limit per area by the area swabbed. If the area swabbed is 100 cm2, then the limit per swab is:

Limit per swab = 20.8 µg/cm^2 X 100 cm^2 = 2080 µg

The limit in the desorbed swab sample can be calculated by dividing the limit per swab by the amount of solvent (water) used to desorb the swab. If the amount of water used for desorption is 20 mL, the limit in the desorbed swab sample is:

Limit in desorbed swab sample = 2080 µg/20 mL = 104 µg/mL (or 104 ppm)

If the active were a protein containing 50% carbon, the TOC limit (net of the blank) would be 52 ppm TOC.

Example 2: This example is based on the dose of the *active* for *bulk drug* manufacturing, assuming the

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entire equipment train is shared surface area. It is based on 1/1000 minimum therapeutic daily dose of the cleaned active. [Note: The purpose of this calculation is to illustrate the low TOC levels likely if the carryover calculation utilized the entire bulk active equipment train (excluding dedicated items).]

MinTD: Minimum Therapeutic Daily Dose of the active of cleaned product

MBS: Minimum Batch Size of next drug active made in the same equipment

MaxDD: Maximum Daily Dosage of next drug active made in the same equipment

SF: Safety Factor

Example for products A and B: if

- MinTD = 25 mg (or 25,000 µg)
- MBS of the following *drug active* B = 200 g
- MaxDD= Maximum mass of daily dosage unit of *active* B = 100 mg (or 0.100 g)
- SF = 1000

The limit in the next drug active is calculated by dividing the MinTD by the SF and the MaxDD:

$$\text{Limit in next product} = \frac{25{,}000\ \mu g\ (\text{MinTD})}{1{,}000\ (\text{SF})\ X\ 0.100\ g\ (\text{MaxDD})} = 250\ \mu g/g$$

If the default limit for bulk active manufacturing is 50 ppm, and since this calculated value is above 50 ppm (50 µg/g), the value of 50 ppm will be used for subsequent calculations.

The MAC is calculated by multiplying the limit in the next product by the MBS:

MAC = 50 µg/g X 200 g = 10,000 µg

The limit per surface area can then be calculated by dividing the MAC by the shared surface area between the two products. Continuing with the same example, the shared surface area is 1,000,000 cm^2. Then, the limit per surface area is:

Limit per area = 10,000 µg/1,000,000 cm^2 = 0.010 µg/cm^2

The limit (mass) per swab can be calculated by multiplying the limit per area by the area swabbed. If the area swabbed is 100 cm^2, then the limit per swab is:

Limit per swab = 0.010 µg/cm^2 X 100 cm^2 = 1.0 µg

The limit in the desorbed swab sample can be calculated by dividing the limit per swab by the amount of solvent (water) used to desorb the swab. If the amount of water used for desorption is 20 mL, the limit in the desorbed swab sample is:

Limit in desorbed swab sample = 1.0 µg/20 mL = 0.050 µg/mL (or 0.050 ppm)

If the active were a protein containing 50% carbon, the TOC limit (net of the blank) would be 0.025 ppm TOC (or 25 ppb). This concentration is not measureable by TOC in cleaning validation samples.

Example 3: This example is based on the toxicity of a *cleaning agent for formulation/fill* manufacturing. It is based on allowing no more than 1/100,000 of the LD_{50} (mg/kg of body weight in an animal model) of the cleaning agent by an intravenous route in the maximum therapeutic daily dose of the next drug product.

LD_{50}:	Lethal Dose for Cleaning Agent
BW:	Body Weight of patient taking product B
MBS:	Minimum Batch Size of next *drug product* made in the same equipment
MaxDD:	Maximum Daily Dosage of next *drug product* made in the same equipment
CF:	Conversion Factor

Example for cleaning agent A and next product B, if:

- LD_{50} = 100 mg/kg
- BW = 60 kg
- MBS = Minimum batch size of the following *drug product* B = 1000 L
- MaxDD = Maximum daily dosage units of *drug product* B = 10 mL
- CF = 100,000

Note that the product of the LD_{50} and the BW, which is then divided by the CF, is sometimes called the ADI (Acceptable Daily Intake). Some companies may calculate the ADI by first converting the LD_{50} to a NOEL (No Observable Effective Level), and then converting the NOEL to an ADI. Either formulation is acceptable and should result in the same ADI value.

The limit in the next product is calculated by multiplying the LD_{50} by the BW and dividing the resultant product by the MaxDD and by the CF:

$$\text{Limit in next product} = \frac{100\ \text{mg/kg}\ (LD_{50})\ \text{X}\ 60\ \text{kg (BW)}}{100{,}000\ \text{(SF)}\ \text{X}\ 10\ \text{mL (MaxDD)}} = 0.006\ \text{mg/mL (or } 6\ \mu\text{g/mL)}$$

Since this value is more stringent than 10 ppm (10 µg/g or approximately 10 µg/mL) cleaning agent solids, this calculated value will be used for subsequent calculations.

The MAC is calculated by multiplying the limit in the next product by the MBS:

MAC = 6 µg/mL X 1,000,000 mL = 6,000,000 µg

The limit per surface area can then be calculated by dividing the MAC by the shared surface area. Continuing with the same example, if the shared surface area is 120,000 cm^2, then the limit per surface area is:

Limit per area = 6,000,000 µg/120,000 cm^2 = 50 µg/cm^2

The limit (mass) per swab can be calculated by multiplying the limit per area by the area swabbed. If the area swabbed is 100 cm^2, then the limit per swab is:

Limit per swab = 50 µg/cm^2 X 100 cm^2 = 5,000 µg

The limit in the desorbed swab sample can be calculated by dividing the limit per swab by the amount of solvent (water) used to desorb the swab. If the amount of water used for desorption is 20 mL, the limit in the desorbed swab sample is:

Limit in desorbed swab sample = 5,000 µg/20 mL = 250 µg/mL (or 250 ppm)

It is likely in this situation that the manufacturer would utilize a more conservative value for measuring the cleaning agent. For example, utilizing a conductivity value of 5 µS/cm would result in a concentration significantly below 250 ppm for most cleaning agents.

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16.0 LIST OF ACRONYMS

ADI: Acceptable Daily Intake

BCA: Bicinchoninic Acid

CAPA: Corrective and Preventive Actions

CBER: Center For Biological Evaluation and Research

CGMPs: Current Good Manufacturing Practices

CIP: Clean-In-Place

COP: Clean Out-of-Place

CPP: Critical Process Parameters

CQA: Critical Quality Attributes

CTP: Critical Process Parameters

DOE: Design of Experiments

ELISA: Enzyme-Linked ImmunoSorbent Assay

EPDM: Ethylene Propylene Diene Monomer Rubber

EU: Endotoxin Units

FEP: Fluorinated Etyhlene Propylene

FMEA: Failure Mode and Effects Analysis

FTA: Fault Tree Analysis

HACCP: Hazard Analysis and Critical Control Points

HAZOP: Hazard Operability Analysis

HPLC: High Performance Liquid Chromatography

ICH: International Conference on Harmonisation

LCD: Liquid Crystal Display

LOD: Limit of Detection

LOQ: Limit of Quantitation

MAC (or MACO): Maximum Allowable Carryover

NIR: Near Infrared

PAT: Process Analytical Technology

PCR: Polymerase Chain Reaction

PETG: PolyEthylene Terephthalate Glycol-modified

PQ: Performance Qualification (or Process Qualification)

PTFE: PolyTetraFluoroEthylene

QA: Quality Assurance

QbD: Quality by Design

QC: Quality Control

QRM: Quality Risk Management

RSD: Relative Standard Deviation

SDS PAGE: Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis

SIP: Steam-In-Place

SME: Subject Matter Expert

SOP: Standard Operating Procedure

TACT: Time, Action, Concentration and Temperature

TFF: Tangential Flow Filtration

TNTC: Too Numerous To Count

TOC: Total Organic Carbon

TSE: Transmissible Spongiform Encephalopathy

WFI: Water for Injection

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