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Process Validation: A Lifecycle Approach

Annex 2: Biopharmaceutical Drug Substances Manufacturing

Process Validation: A Lifecycle Approach Team

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1.0 Introduction

Significant advancements in the design and implementation of effective validation programs have taken place since the adoption of the lifecycle concept recommended by the International Council on Harmonisation (ICH), U.S. Food and Drug Administration (FDA), and European Medicines Agency (EMA). The lifecycle approach is detailed in the 2011 revision of the FDA *Guidance to Industry: Process Validation: General Principles and Practices* and the European Commission's update of *Annex 15: Qualification and Validation, Eudralex – Volume 4* ***(1, 2)*** . Then, in 2016, the EMA finalized the *Guideline on Process Validation for the Manufacture of Biotechnology-Derived Active Substances and Data to be provided in the Regulatory Submission* specifically to address biopharmaceutical drug substances ***(3)***. These documents refined expectations and aligned process validation practices into the three-stage model now practiced by industry and promoted by regulatory agencies worldwide.

PDA discusses extensively the three-stage approach and its implementation in *Technical Report No. 60: Process Validation: A Lifecycle Approach* and *Technical Report No. 60-2: Process Validation: A Lifecycle Approach, Annex 1: Oral Solid Dosage/Semisolid Dosage Forms* ***(4, 5)***. PDA *Technical Report 60-3: Process Validation: A Lifecycle Approach Annex 2: Biopharmaceutical Drug Substances Manufacturing* continues the series by addressing the implementation of process validation in biopharmaceutical manufacturing. It also serves as an update to and replacement for *Technical Report No. 42: Process Validation of Protein Manufacturing* ***(6)***.

A successful validation program is initiated early in the process development lifecycle and continues until the process and product reaches the end of its lifecycle. The program should be founded on a comprehensive corporate policy that defines an organization's expectations and commitment to process validation principles. This policy should define the corporation's quality management philosophy and identify the components of validation, periodic review or requalification timeframes, documentation requirements (including a validation master plan), validation protocols and reports, and responsibilities of key stakeholders within the organization ***(7)***.

Enhanced risk-based approaches, such as quality by design (QbD), recognize the capability of process control strategies to ensure product consistency and prevent or mitigate the risk of producing a poor-quality product ***(8-10)***. To illustrate how specific QbD elements can be applied to the validation of biopharmaceutical drug substances, this technical report discusses the use of quality target product profiles (QTPPs), critical quality attributes (CQAs), material attributes, and critical process parameters (CPPs). It also indicates which risk management tools can be used to identify the attributes related to the product and process and describes the relationships that link the product profile to quality, product, material attributes, and process parameters.

1.1 Purpose and Scope

The concepts presented in TR-60-3 are intended to assist in the design and implementation of globally compliant validation programs to ensure process reproducibility and robustness as they relate to biotechnology-derived, purified protein drug substances. These models are based on the material and practices established in TR-60 and global regulatory guidances. Points to consider are provided to facilitate the collection of data in support of a regulatory filing for the approval of a biopharmaceutical drug substance intended to be used in a pharmaceutical product. The science-based practices provided here are grounded in the experiences of a PDA task force comprising a cross-section of industry professionals and experts in the field. The approaches are intended to add value, support good business practices, and meet current compliance and regulatory expectations.

This technical report focuses on the validation of biopharmaceutical processes used to manufacture therapeutic proteins, polypeptides, and vaccine drug substances. These drug substances are produced from recombinant or nonrecombinant cell-culture expression systems and can be characterized using appropriate analytical procedures. This information also applies to biosimilar products and chemically modified

proteins, including pegylated proteins, antibody-drug conjugates, conjugated vaccines, and other conjugated proteins. Selected principles outlined in TR-60-3 may also apply to other product types, such as proteins and polypeptides isolated from tissues and body fluids, and plasma-derived products. The intent is to provide clear technical guidance for the development and design of a process validation master plan using a risk-based lifecycle approach, and to provide a comprehensive overview of strategies that may be used to validate a manufacturing process or unit operations. Cell and gene therapy products, live-virus vaccines, biopharmaceuticals developed from oligonucleotides, and synthetic peptides are not within the scope of this technical report, though some of the concepts may be applicable.

The strategies discussed here primarily focus on the validation of nonsterile, low-bioburden, well-characterized, protein-based drug substance processes. Specific aspects of process validation that are not unique to protein-based drug substances are not addressed in this technical report. Technical Report 60 covers the topics of clinical manufacturing experience (batch records and production data), process performance qualification (PPQ) protocols, PPQ reports, the transition to continued process verification, process validation enabling systems and technology, knowledge management, statistical analysis tools, and process analytical technology (PAT).

A short discussion of aseptic drug substance processes is included; however, there are numerous other texts that address aseptic process validation more thoroughly. Validation, as it relates to reprocessing, reworking, engineering and design qualification, cleaning, shipping, or drug products, are also out of scope, as is process analytical technologies, though it is mentioned briefly.

These topics are covered in-depth elsewhere, including in these PDA technical reports:

- *Technical Report No. 52: Guidance for Good Distribution Practices for the Pharmaceutical Supply Chain* ***(11)***
- *Technical Report No. 54-4: Implementation of Quality Risk Management for Pharmaceutical and Biotechnology Manufacturing Operations, Annex 3: Case Studies in the Manufacturing of Biotechnological Bulk Drug Substances* ***(12)***
- *Technical Report No. 58: Risk Management for Temperature-Controlled Distribution* ***(13)***
- *Technical Report No. 60: Process Validation – A Lifecycle Approach* ***(4)***
- *Technical Report No. 70: Fundamentals of Cleaning and Disinfection Programs for Aseptic Manufacturing Facilities* ***(14)***
- *Technical Report No. 74: Reprocessing of Biopharmaceuticals* ***(15)***

PDA technical reports do not establish mandatory standards for process validation; instead, they are intended to provide a single-source overview that complements existing guidance documents listed in the references section. Consulting the appropriate regulatory authority for agreement on the strategies employed for process validation studies is always advisable.

2.0 Glossary of Terms

Health authorities have repeatedly requested that industry use ICH and regulatory terminology wherever available. Since definitions alone are insufficient to clearly convey concepts, references to source documents are provided; in addition, many of the concepts are more fully explained within this technical report. Current ICH and regulatory authority definitions are used, except when more clarity was deemed necessary by the task force. Where two definitions used in current guidance documents are considered applicable, both are provided. Some regulatory guidelines may offer other definitions that could also be considered.

Process validation lifecycle terminology differs globally. Some terms may be used differently from company to company, and some may be subject to change in the future. An organization must select the terms it will use in its validation program, define them clearly, and use them consistently in both inspection and registration documentation, within the company and in regulatory filings. For the purposes of this technical report, the following definitions are used.

Action Limit
A limit that, when exceeded, indicates a process is outside of its normal operating range. A response to such an excursion should involve a documented investigation and corrective actions based on the results of that investigation.

Alert Level
An established level that, when exceeded, is giving an early warning of a potential drift from normal operating conditions; while not necessarily grounds for definitive corrective action, it typically requires follow-up review.

Acceptance Criteria
Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures which the drug substance or drug product or materials at other stages of their manufacture should meet ***(16)***. Exceeding the acceptable range for a critical parameter during subsequent validation studies may result in questionable product quality that would require initiation of an investigation and possible batch rejection.

Process Characterization Study
A study that evaluates the process to increase process knowledge and examines proposed ranges and their individual and/or combined impact on target protein quality. Process characterization studies include deliberate variation of parameters to determine their effect on product quality attributes, often conducted as small-scale studies. (Also known as process evaluation studies, process justification studies, engineering studies, development studies, robustness studies, or process design studies.)

Contaminants (Contamination)
5Control Strategy
A planned set of controls derived from current product and process understanding that assures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control ***(10)***.

Continuous Manufacturing
At least two process unit operations conducted under predetermined control conditions without process interruptions, where real-time process controls (PATs) may be used to meet the process requirements.

Design of Experiments (DOE) (or Formal Experimental Design)
A structured, organized method for determining the relationship between factors affecting a process and the output of that process ***(8)***.

Design Space
The multidimensional combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality. Working within the design space is not considered as a change. Movement out of the design space is considered to be a change and would normally initiate a regulatory post approval change process. Design space is proposed by the applicant and is subject to regulatory assessment and approval ***(8)***.

Documentation *(18)*
Development Reports
Documentation and description of work done during the early phases of development (Stage 1). The goal is to document information about the way the process works and to document why key choices were made in selecting the specifics of the process (e.g., flow rate or temperature). These documents can serve as a reference during investigations of deviations and during the design of specific validation and process characterization studies.

Process Characterization Report
A report that includes results from a process characterization study with information on the performance of one or several unit operation(s).

The report describes process characteristics, the parameters (e.g., critical or noncritical) and their studied ranges (limits) and may outline acceptance criteria for process performance qualification protocols.

Process Validation Master Plan

A document that defines the process validation scope and rationale for Stage 2 and Stage 3 and contains the list of process validation studies to be performed.

Process Performance Qualification Protocol

A written plan preapproved by the quality unit that specifies critical steps, controls, and measurements. The process performance qualification protocol states how process performance qualification or other validation studies will be conducted, identifying sampling, assays, specific acceptance criteria, production equipment, and operating ranges. Results obtained for each study described in the protocol should be evaluated in an associated process validation report.

Process Performance Qualification Report

A report approved by the quality unit that summarizes specific tests performed, compares the test results with the protocol acceptance criteria, and addresses deviations encountered during the study.

Drug Product (or Dosage Form, Finished Product, Medicinal Product)

A pharmaceutical product type that contains a drug substance, generally, in association with excipients ***(16)***. The dosage form in the final immediate packaging intended for marketing ***(17)***.

Drug Substance (Bulk Material or Bulk Drug Substance, Active Substance)

The material which is subsequently formulated with excipients to produce the drug product. It can be composed of the desired product, product-related substances, and product- and process-related impurities. It may also contain excipients including other components such as buffers ***(16)***.

Impurity

Any component present in the drug substance or drug product which is not the desired product, a product-related substance, or excipient. It may be either process- or product-related ***(17)***.

In-Process Control (or Process Control)

Checks performed during production to monitor and, if appropriate, to adjust the process and/or to ensure that the intermediate or drug substance conforms to its specifications ***(17)*** and/or other defined quality criteria (e.g., limits for bioburden and endotoxin).

Installation Qualification (IQ)

Documented verification that the equipment or systems, as installed or modified, comply with the approved design, the manufacturer's recommendations, and/or user requirements ***(17)***.

Intermediate

A material produced during steps of the processing of a drug substance that undergoes further molecular change or purification before it becomes a drug substance ***(17)***.

Normal Operating Range (NOR)

A defined range, within (or equal to) the Proven Acceptable Range, specified in the manufacturing instructions as the target and range at which a process parameter is controlled, while producing unit operation material or final product meeting release criteria and CQAs ***(5)***.

Ongoing (Continued) Process Verification (OPV)

A formal plan to assure the process remains in its validated state during routine (post-PPQ) production and the process remains in a state of control ***(2, 3)***.

Operational Qualification (OQ)

Documented verification that the equipment or systems, as installed or modified, perform as intended throughout the anticipated operating ranges ***(17)***.

Parameters

Process Parameter

An input variable or condition of the manufacturing process that can be directly controlled in the process. Typically, these parameters are physical or chemical (e.g., temperature, process time, column flow rate, column wash volume, reagent concentration, or buffer pH).

Critical Process Parameter (CPP)

An input process parameter that should be controlled within a meaningful operating range to ensure that drug substance critical quality attributes meet their specifications. Although parameters with wide operating ranges may also impact product quality, they are generally easily controlled and not as likely to result in excursions that impact quality and are therefore low risk of occurrence.

Performance Indicator (or Performance Attribute)

An output variable or outcome that cannot be directly controlled but is an indicator that the process performed as expected.

Performance Qualification (PQ) (or Equipment Verification)

Documented verification that the equipment and ancillary systems, as connected together, can perform effectively and reproducibly based on the approved process method and specifications ***(17)***.

Process Performance Qualification (PPQ)

The second stage of process qualification. It includes a combination of the actual facility, utilities, equipment, and trained personnel and the commercial manufacturing process, control procedures, and components to produce commercial batches. A successful PPQ will confirm the process design and demonstrate that the commercial manufacturing process performs as expected. Batches prepared are also called conformance batches or PPQ batches.

Process Validation

The documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce an intermediate or drug substance meeting its predetermined specifications and quality attributes ***(1, 17)***.

Concurrent Process Validation

Validation that occurs during manufacturing of drug substance for batches that can be released and used in a final drug product for commercial distribution based on thorough monitoring and testing of the drug substance batches ***(1, 17)***.

Proven Acceptable Range (PAR)

A characterized range of a process parameter for which operation within this range, while keeping other parameters constant, will result in producing a material meeting relevant quality criteria ***(8)***.

Qualified Assay

An assay that is not fully validated but is documented to be suitable for its intended use, including sample collection and handling procedures. Such an assay should be demonstrated to be accurate, precise, linear within the range of use, and show no interference from process stream components (i.e., spike recovery).

Quality Target Product Profile (QTPP)

A prospective summary of the quality characteristics of a drug product that ideally will be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product ***(8)***.

Specification

A list of tests, references to analytical procedures, and appropriate acceptance criteria that are numerical limits, ranges, or other criteria for the test described. It establishes the set of criteria to which a material should conform to be considered acceptable for its intended use ***(17)***.

Set Point

The target for a parameter. The range around the set point is commonly stated in the manufacturing procedures or batch records.

Unit Operation (or Process Step)

A discrete step or manipulation in a manufacturing process where process and operating parameters are defined to achieve a specific process objective.

Validation

A documented program that provides a high degree of assurance that a specific process, method, or system will consistently produce a result meeting pre-determined acceptance criteria ***(17)***.

2.1 Abbreviations

CCS Container Closure System
CMC Chemistry, Manufacturing, and Controls
CPP Critical Process Parameter
CQA Critical Quality Attribute
DOE Design of Experiment
FMEA Failure Mode Effects Analysis
IQ Installation Qualification
OPV Ongoing Process Verification
OQ Operational Qualification
PAT Process Analytical Technology
PPQ Process Performance Qualification/ Process Validation
QTPP Quality Target Product Profile
TPP Target Product Profile

3.0 Stage 1: Building and Capturing Process Knowledge — Process Design

Several aspects of GMP and process development should be completed prior to the process performance qualification/process validation (PPQ) of the manufacturing process. PPQ requires that validated assays, calibrated instruments, and qualified production support systems have already been established with documentation that was completed prior to execution of the PPQ studies. Furthermore, the process should be fully developed, characterized, and documented ***(19, 20)***. These and other specific supporting activities are briefly described in **Sections 3.1-3.6**.

In *Technical Report No. 60: Process Validation: A Lifecycle Approach and Technical Report* (TR-60), Figure 3.0-1 illustrates the overall sequence of activities during Stage 1. At this stage, the quality target product profile (QTPP) and the critical quality attributes (CQAs) are defined, and an initial risk assessment is performed to categorize process parameters. This risk assessment drives the selection of parameters for process characterization studies. These studies confirm or eliminate parameters from the critical classification and assist in establishing ranges.

Fundamental to a lifecycle approach to process validation is process knowledge, as well as full understanding of the facilities, equipment, and technology to be used in the process. The process must show consistency and be well-defined, controlled, and reproducible. Input parameters and performance (output) parameters should be identified, and appropriate operating limits or acceptance criteria should be defined. Process-characterization studies performed during process development and scale-up contribute to establishing this process knowledge database, along with historical information on clinical trial lots manufactured and other knowledge that may be available on the process or similar processes.

Final written development and characterization reports should include discussion, justification or rationale, details of the study, resulting data, recommendations, and cautions. These reports should be internally approved and controlled according to company standard operating procedures (SOPs) ***(21)***. Regulators may review development documentation containing the justification for parameter ranges. PPQ lots usually focus on process performance run at the planned set-point of each operating parameter range. Since justification of the operating range for each operation may not be demonstrated during PPQ, the ranges should be justified using appropriate process-characterization studies (or technical rationale, where not possible) which, for practical reasons, are usually done using scale-down models.

Central to defining the process is a documented change control program, which should be in place throughout clinical product development ***(22)***. Change control ensures that proposed changes are justified, examined, and documented so the process is not altered without a thorough review and

proper documentation ***(17)***. Information about changes during development may affect the product application review process, as some clinical trials may have been conducted with material made prior to a change that took place during development.

Although formal process validation is not generally required in Phase 1 of clinical development, some exceptions are made for product safety issues (e.g., viral clearance validation for mammalian cell products). After Phase 1 and the decision to continue product development, efforts to develop a commercial process must ensure that the process meets all validation requirements. During the commercial development phase, more detailed process knowledge should be collected that describes critical and noncritical parameters, operating ranges, and product impurity profiles as a function of each individual unit operation and the process as a whole ***(23)***. This information, together with the history of the process, should be captured in a series of development reports or process-characterization reports. These documents will serve as the repository of key development information and are critical to assembling subsequent process validation plans and protocols, as well as providing ancillary process support during preapproval inspections.

Numerous deliverables, described in TR-60, are the output of Stage 1. These development deliverables should be documented in accordance with internal development policies and procedures and should be fully traceable (i.e., traceable to raw data). The documents should provide a history of processes and process changes with supporting data, which should include commercial batch records that will be used for the PPQ.

3.1 Sequence of Activities Leading Up to Stage 2 – Process Performance Qualification

While the initial focus of process development may not be to develop a fully validated process, many of the activities integral to developing a robust, reliable process necessitate an awareness that process qualification may be an end goal. Many of these processes may remain constant throughout development; however, others will change during the various stages of scale-up and process optimization. With each incremental change, an overall awareness of the ability to validate the process is of primary importance.

These activities include, but are not limited to:

- Molecule selection
- Host cell system/cell line selection
- Identification of all raw materials
- Cell culture/fermentation process development
- Harvest process development
- Purification process development
- Definition of step yields and other process outputs
- Identification and development of all filtration systems
- Formulation development and product presentation
- Container closure system (CCS) selection
- Shipping qualification of the final drug substance containers
- Analytical method development
- Testing, sampling, and in-process controls
- Toxicology/pharmacokinetic studies
- Definition of process scale
- Process characterization

3.2 Analytical Methods

Analyses of product and process stream characteristics are an integral part of the process characterization and validation exercise. Demonstrating an effective and reproducible process relies on the avail-

ability of appropriate assays. The level of assay qualification and validation increases as the product proceeds through the clinical trials.

Process validation studies are designed to explore process characteristics in more depth and detail than the studies applied during routine drug substance manufacture. Assays, additional to those used for normal in-process monitoring and lot-release testing, are often used during the execution of process validation studies. Assays should be chosen to demonstrate that each process step (unit operation) is performing as designed. Some examples of these assays are provided in **Table 3.2-1.** (**Note:** This table is not all-inclusive and does not cover product-specific assays.)

Table 3.2-1 Examples of In-Process Test and Lot Release Assays†

Assay	Application/Purpose
SDS PAGE* or CE-SDS methodology	Lot release, identity, purity, in-process control Used only during PPQ, purity (in-process product streams)
Carbohydrate analysis	Lot release, identity, consistency Characterization (in-process product streams)
Isoelectric focusing	Lot release, purity Characterization of charge forms
Peptide mapping	Lot release, identity Characterization (involves more detailed analysis of the peptide map, usually by mass spectrometry)
Protein concentration (A280 absorbance, HPLC**)	Lot release, quantity, in-process control (drug substance and intermediates)
Biological activity assay (or potency assay)	Lot release, activity, in-process control
Size exclusion chromatography	Lot release, purity (aggregated species), in-process control Used only during PPQ of in-process product stream (aggregate formation and clearance)
Reversed phase HPLC	Lot release, identity, purity, in-process control
Ion exchange chromatography or CE methodology for charge heterogeneity	Lot release, identity, purity, in-process control
Endotoxin	Safety
Bioburden or sterility	Safety
Mycoplasma	Safety
In vitro adventitious virus testing	Safety
Host cell protein (ELISA and LCMS profiling for identification)	Process clearance, scaled-down spiking studies, used only during PPQ (in-process product stream)
Host cell DNA	Process clearance
Process impurities	Process clearance of cell culture components, column leachates, etc.

† This table is not exhaustive. Additional product specific analytical methods may be required depending on the properties of the molecule

* SDS PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis

**HPLC: high performance liquid chromatography

Additional nonroutine analytical methods used for characterization purposes must be adequately qualified or scientifically justified for their intended purpose prior to use in process validation so results are dependable and unambiguous.

Qualification of nonroutine methods should define the limits of detection and quantification, as well as assay precision, in the same sample matrix that will be tested during process validation. Appropriate assay controls and/or standards should also be chosen to help ensure proper assay performance, and the qualification should be documented in a method qualification report. These assays may be used with proper notebook documentation in a development laboratory, provided that sample tracking, data integrity, data archiving, and retest policies are in place. An exception to this approach is the use of virus control assays during process validation, which are expected to be fully validated. Nonroutine testing can provide useful baseline data to support future comparability exercises related to process changes. Compendial tests are not typically validated but must be verified according to the respective guidelines of the laboratory in which they are performed ***(24)***.

Reference standard development using material obtained from a representative process is an essential part of Stage 1; it ensures analytical assays perform consistently and makes bridging studies possible when process changes are made. With rapid development and introduction of new biological products, primary reference standards are not likely to be available as official reference materials from a standards organization. The manufacturer should establish an in-house primary reference standard and secondary working standard program. The initial primary reference standard is usually a selected lot of manufactured drug substance analyzed by routine release methods that is additionally characterized by nonroutine assays. Consideration should be given to using this material in nonclinical studies or early clinical studies, in order to begin establishing a product profile associated with safety and efficacy. In addition, reference standards for product- and process-related impurities may also be established for use in the PPQ phase ***(16)***. Stability testing should be part of reference standard monitoring.

Equipment used for assay validation and further application in PPQ and for commercial stage testing should be adequately qualified based on a risk evaluation. Calibration and maintenance programs should also be in place ***(25)***.

New analytical technology, and modifications to existing technology, are continually being developed and should be used when appropriate. As part of the analytical method and product lifecycle, all changes to methods should be evaluated and revalidated as appropriate. In that case, the procedures detailed in the FDA *Guidance for Industry: Analytical Procedures and Methods Validation for Drugs and Biologics* and ICH *Quality Guideline Q2(R1): Validation of Analytical Procedures: Text and Methodology* should be applied to ensure the method remains in the same control ***(26, 27)***.

3.3 Risk Assessments

To execute process validation effectively, risk assessments should be used throughout all stages, beginning at Stage 1. The types and number of risk assessments and experimental studies conducted during process development depends significantly on the nature of the manufacturing process and the types of unit operations employed. A variety of risk assessments can be applied to guide empirical studies and decisions regarding control strategy design. ICH *Quality Guideline Q9: Quality Risk Management* and *A-Mab: A Case Study in Bioprocess Development* provide examples of the types of risk tools that can be employed ***(9, 28)***. An initial risk assessment is conducted for each unit operation by examining the associated parameters and determining potential for their impact on preliminary or known CQAs. Ishikawa diagrams, failure mode and effects analysis (FMEA), or cause-and-effect matrices are useful tools for this purpose. This assessment identifies those process parameters that require further study. Scientific and engineering expertise, platform knowledge, or process experience can be applied to rationalize decisions and provide justifications for the initial risk assessment. The output of this assessment will guide process development activities and prioritize the process steps and parameters for further optimization and characterization. One of the most important risk assessments conducted during Stage 1 determines potential CPPs.

Prior to executing process validation, a quality risk assessment should examine, for example, the:

- Impact on CQAs of all unit operations in the process
- Steps required for viral clearance, e.g., solvent treatment, virus filtration
- Reduction or removal of bioburden and endotoxins, e.g., filtration, in-process holds
- Impact of cell culture and fermentation conditions on posttranslational modifications, e.g. glycoform distribution
- Product purity, e.g., chromatographic purification, filtration, and evaluation of process/product-contact materials for compatibility and leachables ***(29)***
- Product integrity, e.g., product CCS for drug substance
- Product stability, e.g., process time for time-sensitive procedures, storage time, and transport of in-process materials
- Product homogeneity, e.g., mixing

A more detailed discussion of risk assessment can be found in various publications, such as PDA TR-54-4 and TR-60 and ICH Q9 ***(4, 9, 12)***.

3.4 Platform Technology Application during Development

Knowledge from platform manufacturing processes is relevant to new products produced using the same platform. In Stage 1, it may be used to contribute to process knowledge for the new product made using the platform approach. In these cases, the molecule produced by the process may differ only in certain aspects, such as the IgG subclass for a monoclonal antibody or the sequence and posttranslational modifications of the molecule. Some examples of platform technology for protein processes include:

- Cell bank production procedures for cell lines that differ only in the clone and gene of interest for the desired product
- Cell-culture or fermentation strategies for similar cell line platforms (e.g., *E. coli* or CHO)
- Harvest operations for similar fermentation processes
- Resins and chromatography steps that use the same buffers, column equipment, regeneration, and cleaning
- Raw materials used in the process that may be similar or the same
- Ultrafiltration or diafiltration steps using the same filters and similar buffers and equipment
- Viral inactivation steps, such as low pH inactivation
- Viral removal steps, such as nanofiltration
- Filling of bulk material and storage conditions

In cases where data exist for similar processes, gap analyses and risk assessments may be used to determine the impact of differences and the impact on the process characterization that may be needed to address these differences. Justification for using existing data should be included in the development and validation protocols and reports.

3.5 Quality Target Product Profile and Critical Quality Attribute Assignment

The intended quality of the drug substance should be determined through consideration of its use in the drug product as well as from knowledge and understanding of its physical, chemical, biological activity, and microbiological properties or characteristics. These elements can influence the development of the drug product. The QTPP, potential CQAs of the drug product (as defined in ICH *Quality Guideline Q8(R2): Pharmaceutical Development*), and previous experience from related products can help identify potential CQAs of the drug substance. Knowledge and understanding of the CQAs can also evolve during the product's lifecycle ***(8)***.

Manufacturing process development should include, at a minimum, the following elements:

- Listing potential CQAs associated with the drug substance so that the characteristics having an impact on drug product quality can be studied and controlled
- Defining an appropriate manufacturing process
- Developing a control strategy to ensure process performance and drug substance quality

Before any development work can begin, predefined product objectives must be established. Basic knowledge should be obtained on the therapeutic indication, intended patient population, design features of the drug substance, dosage form properties (description, CCS, and strength), route of administration, delivery system, and other quality requirements necessary to meet the patient's needs. This information is described in the QTPP.

Once the QTPP is defined, the next task is to determine which attributes provide essential knowledge to guide process development and characterization studies that, in turn, inform the control strategy. The complex molecular and higher-order structural properties of biopharmaceutical products makes the identification of CQAs challenging since many of these features can impact bioactivity and/or other pharmacological properties (e.g., safety, efficacy, immunogenicity, pharmacokinetic/pharmacodynamic). Manufacturing conditions, handling, storage, and distribution can influence the physicochemical properties of the molecule and contribute to the inherent heterogeneity of the product. Extensive analytical and biological characterization studies are conducted on the molecule to understand the nature of amino acid variants that result from the manufacturing production process, handling and distribution, or change of stability. Modifications to higher-order structural elements and the potential for native or nonnative assembly need to be considered, as does the impact to any chemical moieties added to the protein, either naturally, during cell culture (e.g., glycosylation), or synthetically (e.g., pegylation). Finally, consideration must also be given to the potential impact of process-related impurities, excipients, and CCS that may change molecular properties.

Drug substance CQAs typically include those properties or characteristics that affect identity, purity, biological activity, safety, and stability. When physical properties of the drug substance are important, they can be designated as CQAs. In the case of biotechnological and biological products, many of the CQAs of the drug product are associated with the drug substance and, thus, are a direct result of the design of the drug substance or its manufacturing process. Impurities are an important class of potential drug substance CQAs because of their potential impact on drug product safety. For biotechnological and biological products, impurities can be process-related or product-related. Process-related impurities include cell substrate-derived impurities (e.g., host cell proteins and DNA), cell culture-derived impurities (e.g., media components), and downstream-derived impurities (e.g., column leachables). Determining CQAs for these products should also consider contaminants, as defined in ICH *Quality Guideline Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products*, including all adventitiously introduced materials not intended to be part of the manufacturing process (e.g., adventitious viral, bacterial, or mycoplasma contamination) ***(16)***.

Risk assessments can be performed to rank or prioritize quality attributes. Prior knowledge can be used at the beginning of development, and assessments can be updated periodically with development data (including data from nonclinical and clinical studies) during the product lifecycle. Knowledge regarding mechanism of action and biological characterization, such as studies evaluating structure-function relationships, can contribute to the assessment of risk for some product attributes. There are multiple approaches for assessing attribute criticality, but most involve application of risk-ranking and filtering tools that consider severity as the multiplication product of impact and uncertainty. Impact evaluates potential effects on safety, immunogenicity, efficacy, and other pharmacological properties, while uncertainty considers the reliability of the scientific evidence supporting the impact assessment. Quality attributes are ranked according to the degree of severity relative to a criticality continuum (i.e., not a simple yes-or-no decision) that more accurately reflects the complex structure-function relationships associated with biopharmaceutical products and the inherent difficulty of making classifications with

absolute certainty. The A-Mab Case Study includes details and examples of the tools and approaches involved ***(28)***. Any tool that is developed to determine attribute criticality will include a numerical scoring system that is a component of the overall risk assessment used to develop the final control strategy.

At early stages of process development, limited information may be available to definitively assign criticality levels to all attributes. In that situation, prior knowledge or platform knowledge for related molecules can be applied and CQAs can be classified as potential. As specific knowledge from process and clinical development is obtained, the assessment is informed by in vitro studies as well as by nonclinical and clinical data. Therefore, the severity assessment to determine CQAs is a process conducted multiple times during Stage 1. Importantly, the severity assessment should not be influenced by process capability (occurrence) or measurement capability (detectability), which are addressed with separate risk assessments tools when process development and characterization work proceeds.

Given the types of data that must be evaluated in order to properly define the QTPP and determine CQAs, these assessments must be conducted in collaboration with subject matter experts outside of the core CMC group (i.e., toxicology, clinical pharmacology, pharmacokinetic/pharmacodynamic, and medical). Sufficient understanding of CQAs is essential because process development and characterization studies are focused on this information. All attributes, regardless of assigned criticality, must ultimately be accounted for in the overall control strategy. Whatever tool is used to determine CQAs, the process must be documented and the associated decisions related to attribute criticality must be transferred to the manufacturing organization and justified in regulatory license applications.

3.6 Control Strategy

One primary objective of Stage 1 process validation is to develop a robust manufacturing process along with an appropriate control strategy that ensures consistent product quality. **Section 3.6** provides a basic overview of control strategy development for a biopharmaceutical product. For additional details on the concepts, ICH Q8(R2), Q9, and *Quality Guideline Q11: Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/Biological Entities)* guidelines ***(30)*** and PDA TR-60, should be consulted.

The accumulated product and process knowledge acquired in Stage 1 is used to construct the control strategy that is qualified in Stage 2 and then implemented for the commercial process. Science- and risk-based approaches provide the basis for rationalizing the most appropriate controls to ensure product quality based on the defined CQAs. Risk assessments and process characterization studies can be performed in parallel and are often iterative to support parameter classification and development of the control strategy. The following control elements can be used either individually or in combination, depending on the determined risks:

- Raw material controls
- Procedural controls (e.g., hold times)
- Process parameter controls (set points, ranges, CPPs)
- In-process testing (including methods and acceptance criteria)
- End-product (release) testing (including methods and acceptance criteria)
- Characterization testing
- Process monitoring
- Engineering (facility, instrumentation) controls
- Stability

End-product testing is only one part of the control strategy, and it may not be necessary to conduct end-product testing (i.e., release specification) on all CQAs. For example, host cell proteins (HCPs) and DNA process-related impurities are classified as CQAs due to their potential to impact patient safety. Extensive process development and characterization studies can generate sufficient data to demonstrate adequate

and consistent clearance. Therefore, this process understanding could be used to justify that parametric controls would provide sufficient control elements without the need for routine end-product testing.

3.6.1 Risk Assessment to Support Control Strategy Development

Process development studies inform the impact a process parameter may have on CQAs. A risk assessment is performed to determine the extent of the risk from the process parameters and drives the development of the control strategy. One method to determine the level of risk and develop mitigation strategies is the use of an FMEA. Using this method, the control strategy risk is reflected in the overall risk score (severity x occurrence x detectability = risk priority number (RPN)). Where the overall score indicates that elements of the process may be vulnerable, appropriate control elements may be applied to lower the risk of occurrence or to manage the level of detectability (e.g., in-process testing, parametric control, end-product testing).

After the control elements are included in the process, further process characterization studies are conducted to assess the effectiveness of the control strategy. Further refinement may be undertaken based on those results, and then the overall level of risk can be reassessed.

Prior to conducting a risk assessment, a number of prerequisites provide the data to make informed decisions concerning risk:

- Quality attributes criticality assessment (CQAs)
- Knowledge of functional relationships between process inputs (process parameters and material attributes) and outputs (typically established through experimentation and supported by data)
- Understanding of site manufacturing technology and equipment control capability
- Development of analytical methods capable of detecting impacts to CQAs

Section 7.0 (**Appendix 1: Example of Failure Modes and Effects Analysis**) provides an example of an FMEA and the subsequent RPN assigned for the control strategy design.

3.6.2 Critical Process Parameters

In accordance with ICH Q8(R2), a CPP is a parameter whose variability has an impact on a CQA and therefore should be controlled and monitored to ensure the process produces the desired quality. Where data or inference suggests that a process input parameter has the potential to impact CQA(s), the parameter is classified as a CPP. Although redundant controls or immediate detection can reduce the likelihood of a parameter being outside of the normal operating range, the magnitude of the output response (severity of impact) is the sole determination of parameter criticality. "The fact that a risk of failure is mitigated by applying a robust proactive control strategy should not allow for underestimation of criticality" ***(31)***. Accumulated knowledge on biopharmaceutical production processes has led to a recognition that certain unit operations and their associated parameters that could affect a CQA related to safety will, in all likelihood, be assigned as critical. For example, in a low pH viral inactivation step, the pH and the incubation time will most likely be assigned as critical parameters since these have direct impact on the amount of viral inactivation. These types of parameters, irrespective of the level of control, should be considered CPPs, unless data is available to demonstrate they do not impact CQAs. Typical examples of other unit operations are provided in **Section 9.0** (**Appendix 3: Unit Operations Used in Biopharma Processes**).

3.6.3 Noncritical Process Parameters

In biopharmaceutical processes, multivariate and univariate experimentation often reveals that the impact of deliberate variation in process inputs is not limited to CQAs. Noncritical process parameters may influence process performance or noncritical process outputs that may be a measure of process consistency. In-process testing is routinely conducted in drug substance manufacturing to monitor process indicators (e.g., titer, chromatography yield, processing time). These indicators, although not critical, can be sensitive to gradual drift or process changes that may not be immediately apparent in drug substance CQAs. Although **Table 7.0-1** in **Appendix 1** shows only CPP and non-CPP cat-

egories, some manufacturers further differentiate non-CPPs and others, which need to be explained. When used, any additional subclassifications should be clearly outlined in written documentation as part of the quality management system.

3.6.4 Process Characterization

Process characterization comprises a set of documented laboratory studies in which parameters are purposely varied to determine their effect on product quality attributes and process performance. Parameter ranges that are explored during process characterization are generally wider than those anticipated during routine manufacturing and may be chosen based on a combination of equipment design capability studies and previous manufacturing history. Experimentation can be conducted using univariate and multivariate studies, as appropriate. Whenever possible, design of experiment (DOE) approaches should be used to understand interactions of multiple parameters. The use of single-factor OFAT (one factor at a time) studies could be considered to identify parameter sensitivities potentially confounded by other parameter interactions. In addition to evaluating single-unit operations, consideration should be given to studying multiple-unit operations using linkage studies. Scale-down models of unit operations are essential components of process development. The suitability for intended purpose of all scale-down models and the predictive capability relative to larger-scale production (e.g., clinical, commercial) must be demonstrated, justified, and documented. Statistical methodology can be applied to establish equivalency of small and large operational scales; however, the size of the dataset is a practical consideration. DOE methodologies are often used to optimize experiments, explore the effects of parameter interactions, and apply statistical rigor in range selection ***(32)***. Determination of CQAs (product quality attributes that affect product safety, identity, strength, quality, and purity) precedes process characterization. Parameters that affect CQAs or operational reliability should be explored in this phase of work. Process characterization studies should be completed prior to writing process validation protocols. Although formal approval from the quality assurance unit may not be required, process characterization studies should be conducted following good scientific documentation practices and the study results documented as part of the technical reports.

Results of the process characterization studies support parameter ranges used during the process validation studies. Understanding the effect of parameters on product quality attributes can also support increased flexibility consistent with continuous improvement efforts.

Often, parameters that have significant effects on product quality attributes are identified during early process development studies or manufacturing campaigns. Examples of parameters and their classification are given in **Section 9.0**.

Process characterization is a risk-based activity, part of an overall quality risk management process. The first step in the process is identifying risk of the parameters to be studied. During the initial phase of process characterization, a list of presumed high-risk parameters to be studied for each unit operation is confirmed. This initial phase of characterization may eliminate some parameters from the list. In later characterization studies, the high-risk parameters and the applicable ranges that can be set to assure no impact on CQAs are studied and determined. Following confirmation of parameters, detailed process characterization experiments can be designed to collect data for additional information. Because the results of these experiments are used to characterize the process and define acceptable parameter ranges, preapproved study acceptance criteria are not usually included.

Process characterization experiments may be performed using scale-down models that have been demonstrated to accurately represent the full-scale unit operation and the parameters being tested. Ranges for each parameter are investigated to determine if it is noncritical or critical. Measured outputs generally include CQAs and process performance indicators. Critical product quality attributes might include product potency, glycoform distribution, residual reactant or levels of aggregated or clipped forms of the target protein. Process performance indicators might include such measurements as concentration,

yield, or cell mass/growth rate and may demonstrate consistency and robustness of a process ***(19)***. Based on study results, the parameter classifications (i.e., noncritical or critical) are confirmed or disproven.

Study results should be summarized in a series of process characterization reports. The reports should list noncritical and critical parameters, the basis for their determination, and acceptable ranges (with justification for those ranges), as well as any other pertinent information derived from the study.

The concept and principles of process characterization can be applied to all unit operations in the manufacture of therapeutic products—from thawing the working cell bank to distributing the packaged drug product.

3.6.4.1 Design of Experiments

Design space of one or more unit operations is ideally defined by a statistical approach using the DOE method. DOE is a structured, organized methodology for determining the relationships between factors affecting a process and its outputs that helps optimize the process control strategy. The most commonly used software for designing experimental matrices also interprets the data. DOE approaches can be used in the early design stage of the process to identify critical parameters as well as during the late-stage process characterization studies to identify interactions between parameters.

3.6.4.2 Scale-Down Models

Process development typically begins in laboratories at small scale, and small-scale studies continue throughout the product's lifetime, even when it is manufactured at large scale. Process development, process characterization, and process support studies (satellite runs) are performed at small scale. Over the drug product's lifecycle, scale-down models are continuously improved. Most of the initial process knowledge used in regulatory filings is from small-scale data, as very few manufacturing runs are required to generate sufficient material for a clinical trial.

Process validation and support activities for biopharmaceuticals also rely heavily on small-scale studies, especially during process characterization. Process characterization investigates the links between process outputs and intentionally varied inputs and usually employ statistically designed experimental plans. Due to the number of parameters that may be examined, these studies are almost exclusively completed using scale-down models. The validity of these controlled studies depends greatly on the accuracy of the scale-down models employed, and using verified scale-down models to complete the process characterization studies to ensure high quality data is recommended ***(30)***. Any bias or offsets between the scale-down model runs and manufacturing-scale data should also be understood so that this information can be factored into the data analysis and interpretation. An example of a scale-down model qualification is provided in **Section 8.0** (**Appendix 2: Example of Scale-Down Model Qualification**). Throughout the product's lifecycle, the model should be improved by verifying comparability with additional data from a larger number of manufacturing-scale runs.

To reduce potential variability between the scales, input materials from manufacturing, such as media, buffer, and in-process product pools, should be used for the small-scale experiments to the extent possible. The materials used in the scale-down studies should include chromatography resins, filters, membranes, reagents, and solutions that meet the same specifications used in manufacturing. The acceptance criteria must include relevant product quality attributes, performance indicators (e.g., step yield), and other step-specific characteristics (e.g., metabolic profiles, chromatograms). Results from the different scales should be comparable and meet the predefined criteria; however, if there are differences, the differences must be explained explicitly, and the rationale for the model's acceptability must be given ***(1)***.

The validation of process consistency must occur at commercial scale using the intended facility and equipment. However, the validation activities consist of a combination of commercial-scale and scale-down studies, depending on the appropriateness of the model being used and the nature of the study.

While performing studies at commercial scale is preferable, manufacturing limitations require that small-scale studies be used. These limitations can be due to time and resources (experiments at commercial scale are very expensive) or may relate to the practicality of performing studies at scale (i.e., viral clearance). Viral and impurity clearance studies, cell bank qualification studies, resin and membrane lifetime studies, and chemical stability studies for buffers, reagents, and process intermediates are validation activities typically completed at small scale. While resin and membrane lifetime studies can be completed at small-scale, a confirmatory, protocol-driven study is often required in manufacturing (see **Section 4.2.2.8**). Studies for limit of in vitro cell age are conducted at pilot scale or full scale ***(33, 34)***.

Process validation also includes in-process intermediate holding steps. Process intermediate stability studies can be done either at full scale or in a scaled-down system and are often done using a combination of the two. Microbial ingress prevention (bioburden and endotoxin) must be proven using appropriate manufacturing vessels (see **Section 4.2.2.1**).

3.6.4.3 Establishing Process Parameter Ranges

Sources of common-cause variability need to be understood, and this knowledge must be documented and transferred to the commercial manufacturing site responsible for validation and maintenance of the validated state throughout the product lifecycle. Input materials (e.g., raw materials used in cell culture medium and excipients) are known to introduce variability in drug substance quality attributes. Therefore, multiple lots of raw materials and excipients need to be evaluated in development studies and further represented in the clinical manufacturing batches. Stage 1 activities also provide an understanding of how process parameters at each unit operation can introduce variation in drug substance quality attributes. Process development and characterization studies are designed to generate the needed information to define parameter impact on drug substance quality attributes. This product-specific information can be combined with platform knowledge from related molecules, manufacturing experience, and historical data to define appropriate setpoints and associated operating ranges. The use of contour plots and/or prediction models is an effective way to summarize these details in regulatory licensing applications.

Accelerated regulatory pathways (e.g., U.S. breakthrough therapy designation, EMA's PRIME, Japan's SAKIGAKE) may result in situations where only a few batches have been manufactured prior to executing validation. In these cases, the understanding of process variability will be limited, and platform knowledge must be leveraged to set suitable criteria for process parameters, which can be refined as more product-specific process knowledge becomes available. Even in cases where sufficient production experience is available prior to validation, it is unlikely that all sources of variability are completely understood or will match exactly the variation to be experienced when, after product approval, multiple manufacturing campaigns are executed over many years. To account for this uncertainty in future process variability, the manufacture of some clinical trial batches using process parameter settings outside of typical setpoints, but within established ranges, is advisable. This approach ensures that the variation in product quality attributes introduced by the process is represented in clinical studies.

3.6.5 Criticality Assessment of Raw Materials

A material qualification program should be in place for raw materials that will be used for the initial manufacturing. At a minimum, vendors should be selected, and specifications should be established, especially for noncompendial-grade materials. As the manufacturing evolves through clinical trials, a more complete program should be defined that includes systems to qualify new materials or alternate vendors, responses to material discrepancies, material stability, vendor audit requirements, and supplier sustainability ***(35)***. A raw materials control program should be well established prior to proceeding with PPQ. Early in the design stage of development, raw materials should be selected using a defined process, taking into consideration multiple factors regarding the source and vendors, including:

- **Compendial status of the raw material –** Compendial raw materials should be used where available. Consideration should be given to sourcing multi-compendial materials (i.e., meeting U.S., EU, and Japanese requirements) for a product that will be distributed globally. Any material

that will be an excipient in the drug product is required to be a compendial-grade material.
    - For materials that cannot be sourced as compendial grade, the manufacturer is responsible for defining the appropriate tests and specifications.

- **Vendor selection –** Raw materials from qualified vendors should be used. This requires early review of the status of raw material vendors during the design stage to assure all vendors used for commercial manufacture will meet the quality system requirements of the company. All vendors should be qualified by the time of the PPQ.
- **Identification of multiple sources for raw materials –** Often raw materials shortages and vendor problems arise during the life of a product. Having more than one source of raw materials identified and included in the process validation exercise is prudent for highly variable materials (e.g., soy-based media). An approach to including materials from different vendors in the PPQ will ensure that all identified raw materials are suitable.
- **Source and origin –** Wherever possible, the use of nonanimal-sourced or nonanimal-derived materials is preferred (e.g., biochemically- or synthetically-produced). The source and origin for each raw material from each vendor should be reviewed and documented on an ongoing basis. Certificates of suitability and certificates of compliance with procedures to prevent contamination with adventitious agents, and transmissible spongiform encephalopathy (TSE) agents where appropriate, should be reviewed and found adequate ***(36)***. When animal-derived sources are required, such as bovine serum, use of irradiated and properly tested serum sourced from countries at a low risk for TSE should be used. For adherent cell processes, alternate sources of nonanimal-derived trypsin should be evaluated and used where possible. In addition to TSE concerns, source materials used for cell culture (including plant-based materials) should also be tested for the presence of *Mycoplasma* ***(37, 38)***.
- **Risk assessments –** A risk assessment process should be undertaken to define which raw materials are considered critical for the consistent performance of the process and which may have a potential impact on the CQAs of the drug substance. Critical raw materials controls should address potential risks to product quality attributes. See ICH *Quality Guideline Q3D(R1): Guideline for Elemental Impurities* regarding aspects to include in material risk assessments ***(39)***.
- **Critical material attributes –** Once the critical raw materials have been identified, the attributes that are important for product quality should be determined, and testing should be implemented to ensure these attributes are met for each batch of raw material.
- **Testing of critical material attributes –** Testing could include specific performance testing, such as for some resins and media components. A growth promotion and support test for critical mammalian cell culture media to assure the growth performance of the culture, for example, will meet required standards. The decision to include performance testing is made on a case-by-case basis, based on knowledge of the potential variability of the raw materials as determined through the risk assessment process.
- **Consumables –** A quality assessment process should be in place for the many consumables used in the manufacturing process. Such items should be categorized in the risk assessment process and the appropriate level of control testing or inspection should be in place. A discussion of the controls for single-use processing equipment is provided in PDA *Technical Report 66: Application of Single-Use Systems in Pharmaceutical Manufacturing* ***(40)***.

4.0 Stage 2: Process Qualification

TR-60 provides extensive details related to process qualification; **Section 4.0** summarizes the readiness assessment and process performance qualification (PPQ), and other process validation studies. Process

qualification marks the transition from development and clinical batch manufacturing to routine commercial production. The objective of process qualification is to demonstrate that the process works as intended and yields reproducible commercial product. **Figure 4.0-1** outlines the general process for preparing for process validation activities.

Prior to executing PPQ, a readiness assessment should be conducted to determine availability or completion timing of required information and ensure that a facility, equipment, and trained staff are in place to successfully complete the exercise. The assessment should include the process validation master plan, quality system, and the ability to control process variability within the control strategy through appropriate monitoring systems. A risk assessment should be performed as part of the readiness assessment to identify potential issues that should be mitigated or accepted with justification. Representatives from product development, manufacturing sciences and operations, and quality should review the output from the risk assessment, process development data, and supporting documentation. They should also evaluate the capability of the manufacturing facility, equipment, personnel, analytical methods, and laboratories involved in PPQ and other process validation studies. This readiness assessment should determine whether the plant is ready to proceed or if corrective actions are required.

4.1 Readiness

As best practice, a cross-functional team is assembled to address the process validation master plan. Members from validation, process development, analytical development, quality control unit (i.e., quality assurance and quality control), regulatory affairs, manufacturing, and technical operations and/or engineering departments, at a minimum, are assigned to the team.

4.1.1 Equipment and Facilities

For GMP operations, equipment, utilities, and facilities should undergo qualification, which will be documented in the process validation master plan prior to PPQ batches and supporting validation studies (e.g., hold time, cleaning validation, etc.). The qualification package is a compilation of specifications, manufacturer's documentation, and testing protocols performed on both the manufacturer

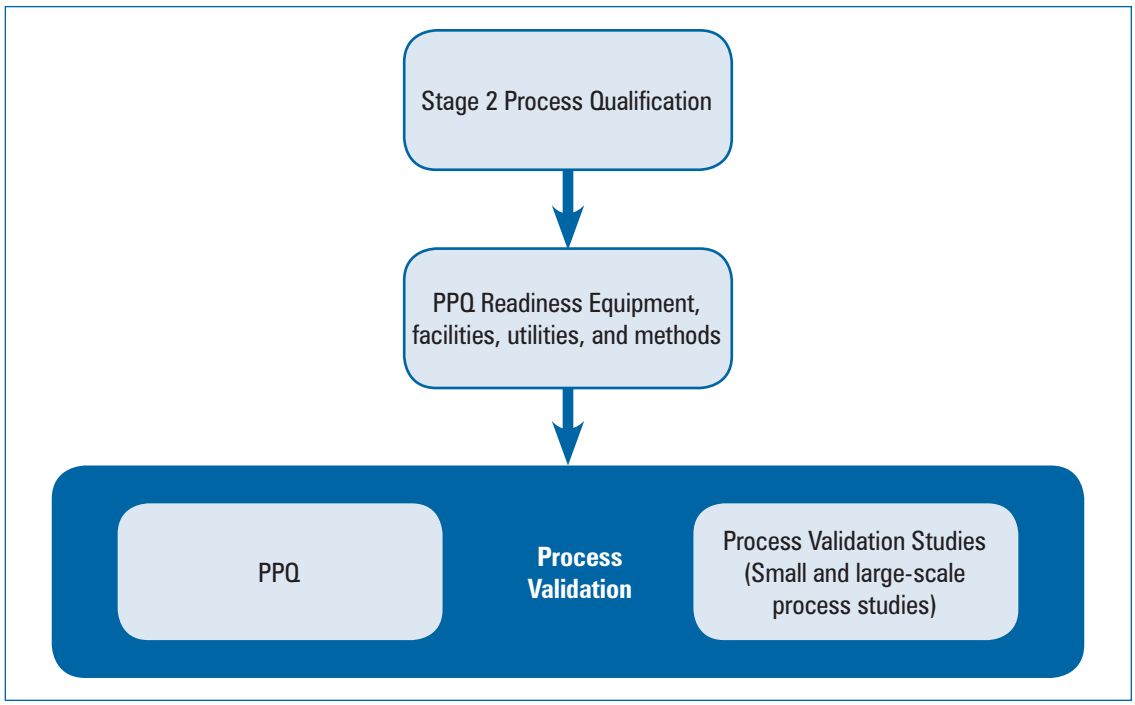

Figure 4.0-1 Activities Leading Up to Process Validation

and user sites. Qualification studies should always be designed and performed on the basis of process parameters and intended usage of equipment. Once the equipment is designed and manufactured according to user requirements, documented testing should be performed to ensure that it is suitable for its intended use. The testing should evaluate equipment operating capability (validated control ranges for parameters), capacity, and safety, as well as an assessment of all process-wetted components for compatibility, durability, and potential leachables.

The use of automation for production-related processes has become a widely accepted practice; when using automated equipment, particular attention should be paid to data integrity controls. Such elements as audit trail, data encryption, access controls, and other relevant factors should be considered in user and design specifications as well as in testing procedures. Regulations and guidances thoroughly describe requirements and best practices to be followed to ensure automated systems data integrity ***(41–43)***.

Qualification lifecycle is predefined in the comprehensive validation project master plan that should be completed before the process can be run and validated ***(22)***. In addition, programs for time-driven qualification status assessment, preventive maintenance, instrument calibration, and change control need to be established. As an example, commissioning input could be employed to achieve a qualified state. Commissioning involves increased engineering participation and, when done thoroughly with quality assurance oversight, can abbreviate much of the conventional installation, operational and performance qualification. The quality assurance unit must be fully involved to ensure that all those participating in commissioning activities are fully trained where commissioning is used to support qualification activities. One aspect of commissioning may be the use of a risk assessment (e.g., FMEA) ***(44,45)***. An in-house engineering department performs and documents the tests and then turns the equipment package and documentation over to the manufacturing and validation units. Approaches for planning and performing qualification studies are well described in PDA TR-60, ISPE's *Baseline Guide Volume 5: Commissioning and Qualification*, and *ASTM E2500–13: Standard Guide for Specification, Design, and Verification of Pharmaceutical and Biopharmaceutical Manufacturing Systems and Equipment* ***(4,45,46)***.

Cleaning validation of product contact equipment parts may be performed concurrently with PPQ batches with cleaning occurring between batches or performed separately along with engineering batches or using soiling technique. Selection and suitability of cleaning agents for product-contact surfaces or processing-aid surfaces (e.g., chromatography resins) must be established prior to initiation of cleaning validation and PPQ. Dirty and clean hold studies should also be performed as part of validation. For processes using multiple equipment units of the same specification (e.g., stainless-steel tanks, chromatography units), a grouping strategy could be used based on worst-case scenarios to minimize validation effort without compromising assurance level. To simplify manufacturing operations, improve contamination controls, and further minimize validation effort, single-use systems may be considered a practical, sound alternative to multiuse equipment ***(47,48)***.

4.1.2 Methods

In-process and lot-release analytical methods that are used during the routine manufacture of commercial product to mitigate risk and/or confirm product quality, should be validated prior to initiating PPQ and supporting validation studies (e.g., cleaning validation). Methods may need to be qualified for the sample matrix being tested, as process validation usually entails nonroutine sample matrices. These assays must be performed in a GMP laboratory following established SOPs. ICH Q2(R1) and PDA *Technical Report No. 57: Analytical Method Validation and Transfer for Biotechnology Products* provide detailed guidance for the validation of analytical methods ***(26,49)***.

4.1.3 Raw Materials

Criticality assessments of raw materials should be reevaluated prior to PPQ as outlined in **Section 3.6.5.**

4.2 Process Validation

Elements of process validation, various approaches to PPQ, and types of validation studies needed to complete a process validation package are the focus of **Section 4.2**. Monitoring PPQ lots is the primary element of process validation to show processing consistency, clearance of contaminants, and the ability to meet predetermined acceptance criteria for CPPs, in-process controls, performance parameters, and CQAs. Additional studies, such as stability of in-process intermediates, process solution stability studies, cell line characterization, and shipping validation are generally performed as separate protocols and can be conducted separately from full-scale PPQ lots. TR-60 provides detailed guidance on developing the sampling plans that are required for PPQ studies.

4.2.1 Process Performance Qualification

Demonstration of consistency is confirmation that developmental expectations for process performance and control are being met and that the manufacturing facility, equipment, and procedures are capable of reproducibly manufacturing a drug substance that meets specifications and product quality attributes. Consistent performance parameters, such as yields of drug substance with appropriate product quality attributes during manufacturing, demonstrate process consistency. Continued monitoring of a subset of the original parameters evaluated during ongoing process verification (OPV) may be required to demonstrate consistency beyond the initial PPQ lots (see **Section 5.1**). The criteria used to evaluate consistency should be described in the PPQ protocol. Emerging technologies, such as process analytical technology (PAT), may provide comprehensive in-process monitoring, which may influence future validation approaches ***(50)***.

Typically, a minimum of three consecutive lots is used to demonstrate consistent manufacturing; however, the number of batches required should be risk-based. PPQ lots should confirm that the process can be run repeatedly within the limits defined by the lab-scale characterization studies. PPQ lots should be consecutive and should demonstrate that the manufacturing unit can consistently execute the process. Passing PPQ lots should be consecutive, but they may not always be sequential. Lots may be separated by a failed/invalid lot(s) when the failure is due to a known assignable cause (e.g., operator or equipment) that is neither process-related nor caused by process variability. A justification for not including failed/invalid lots, even if they are not process-related, should be provided in the final validation reports.

4.2.1.1 Process Performance Qualification Options

The two generally recognized options for PPQ and process validation studies are prospective and concurrent. Prospective validation refers to a study which is planned and completed prior to submission of an application for approval of a new product, or for an approved product made under a revised manufacturing process where revisions may affect the product's quality. In the biotechnology industry, prospective validation is the preferred approach and is the primary focus of this document. All drug substance processes should be validated prior to approval of the drug product. In the case of a change to the drug substance process, the potential impact to the drug product and need for additional validation should be evaluated.

Concurrent process validation refers to validation using data collected during ongoing commercial manufacturing and requires regulatory agreement. These studies are long term and require ongoing monitoring at a heightened level. This option includes the same elements as prospective validation except that replicate runs occur during commercial manufacturing and results are reviewed in conjunction with the product lot release. These concurrent studies, conducted under a defined protocol, may consist of full-scale production under standard conditions with additional sampling and testing (e.g., column or membrane lifetime studies). If sufficient validation data for the range of parameters are available from prospective validation studies (e.g., lab-scale studies), these are generally considered acceptable. The design of concurrent validation studies will depend on the type and extent of data available and any prospective studies performed. Protocols for concurrent validation may include accep-

tance criteria that must be met before the lot under study can be released. Concurrent validation may also be used for medically necessary products, where the manufacture of an adequate number of lots is not possible prior to approval; this would be determined in conjunction with regulatory authorities.

Retrospective validation is no longer accepted as a validation approach. The use of historical data for the purposes of supporting a concurrent validation approach may be acceptable under certain conditions.

4.2.1.2 Unit Operation Validation

PPQ is often divided into validation of individual unit operations, or process steps, rather than the entire process. Unit operations can be validated by characterization of the resultant intermediates to determine if they meet appropriate quality attributes; however, this approach should generally be used in the context of validating the entire process. Validation of an individual unit operation may be acceptable for a process change that only impacts that unit operation, determined by risk assessment and development data). If process variability is estimated using a representative scaled-down model designed for an individual unit operation, the process variability at commercial scale need not be challenged. Each unit operation can be defined in terms of its function and the validation should demonstrate that these functions are consistently achieved. Protocol acceptance criteria are prospectively developed to ensure product quality attributes. When changes to the overall process are contemplated, or when deviations occur, the unit operation or operations affected by the change can be isolated and evaluated by applying risk assessment principles.

Unit operation validation is preferred if the following assumptions hold true:

- Validation is performed at set-point within the operating range
- Samples from intermediates steps pass their acceptance criteria and the drug substance meets release specifications
- Critical hold times between critical processing steps are addressed through validation studies

In evaluating complex processes (e.g., vaccines), if choosing to validate process holds, cumulative worst-case hold times can be used to represent the summation of maximum unit operation hold times under normal manufacturing conditions ***(51)***. Validation of the whole process in the manufacturing of a particular drug substance is accomplished through validation of its individual process steps or unit operations. When validating a process, all of the process steps or unit operations should be of the same PPQ lots to demonstrate that the output of one step indicates the next step may proceed, and that the overall process is consistent.

4.2.1.3 Validation Strategies

To facilitate validation, a grouping strategy may be employed to streamline testing and maximize the applicability of the study. The rationale for a grouping strategy must be science- and risk-based, must support the intended use, and must be documented in the project plan, protocol, or report.

4.2.1.3.1 Matrix (or Bracketing) Approach

A matrix (or bracketing) approach to validation is conducted at the full range or extremes of a process or equipment parameter (e.g., strength, batch size, temperature, pH, density, flowrate, vessel size). Successful testing of the extremes validates the entire range of the tested variables. For example, when a group of different-sized vessels of similar configuration are used in a process, the largest and the smallest vessels are validated. Then, validation of intermediate-sized vessels can be encompassed by this study with adequate justification (e.g., via risk assessment). A detailed illustration of this practice is provided in TR-60.

4.2.1.3.2 Grouping Approach

Grouping validation applies to articles with physical and functional similarities, such as equipment that is identical or similar (e.g., laboratory HPLCs, bioreactors, tangential flow filtration membranes, column housing units, tanks). The degree of similarity should be fully documented. Testing of any

group member may be used to support qualification or validation for other items in an established family. Reduced operational and performance qualification testing, such as verification only, for other group members may be conducted. Grouping validation can be considered for equipment cleaning as well as operations ***(52)***. An example would be a bank of three bioreactors of similar design and size to be used in the manufacture of a single product; one reactor may be validated by three consecutive runs, and the other two may be validated by one confirmatory run each.

4.2.1.3.3 Cross-Site or Platform Approach

The cross-site or platform approach consists of validation performed for a system or process at one site being applied to similar systems or processes (e.g., solution hold times, shipping) at another site. The system in question needs to be similar in performance and capabilities, and the impact on the product needs to be comparable.

4.2.1.3.4 Worst-Case Approach

A worst-case strategy consists of testing a condition or a set of conditions encompassing upper and/or lower processing limits and the circumstances that pose the greatest potential risk of product or process failure when compared to routine operating conditions. Such conditions do not necessarily induce product or process failure.

4.2.1.4 Continuous Manufacturing

Batch processing methodologies have been used successfully in the biopharmaceutical industry for many years. The primary setback for implementing continuous processing for biological processes is the process variability and complexity of products derived from biological systems. Employing control strategies that use PAT and new process techniques that aid in expression, purification, and fill/finish to continually produce quality products, however, can overcome that obstacle ***(53)***.

Continuous bioprocessing consists of a process that has fewer or no hold steps and interlinked unit operations that have significantly smaller footprints compared to a traditional batch process. It can provide manufacturers with added benefits, such as faster development, shorter scale-up, and shorter production times. As the biopharmaceutical industry matures and ventures into multiple therapeutic areas, the need for continuous bioprocessing will increase. The driver for this change, which may play a key role in decision-making, is thought to be multifold: reduced batch sizes due to better-yielding products, reduced capital expenditure on newer facilities, and new therapeutics that do not require numerous drug product batches. The process of economics and sound manufacturing strategies must be considered when implementing continuous processing for a biological product ***(54)***.

Implementing continuous manufacturing processes requires reliable instruments and technologies, which suppliers have taken steps toward introducing. Perfusion and other continuous bioprocessing technologies are becoming significantly less complex, less predisposed to contamination, more regulatory-friendly, and more easily scalable than fed-batch methods ***(54)***. Some of these technologies include alternating tangential flow filtration, inline buffer preparation, periodic counter-current chromatography, and single-pass tangential flow filtration technologies. The increased use of PAT is another key component of a successful continuous process ***(50)***. The use of PAT reduces the need for in-process testing and exerts more process control to improve the robustness of the overall process ***(50)***. Technologies such as near infrared and Raman spectroscopy have been and can be used successfully as feedback and feed-forward controls in biological processes.

Another important component of implementing continuous process is the acceptance and guidance from regulatory authorities; regulators are aware of this transition and seem to welcome it as a positive move toward improving process robustness and product quality. In fact, the U.S. government demonstrated its commitment to new technologies recently by enacting *The 21st Century Cures Act*, which supports the continuous manufacturing of drugs and biological products ***(55)***.

4.2.2 Process Validation Studies

Process validation studies may consist of a combination of full-scale and scaled-down studies, depending on the appropriateness of the model being used and the nature of the study. While performing full-scale studies is preferred, manufacturing limitations may require that small-scale studies be conducted. Process validation studies that are performed in scale-down models should be done with preapproved protocols that contain predetermined acceptance criteria and final validation reports. For example, spiking studies may demonstrate impurity removal that requires a scale-down model study because they cannot be conducted at full, commercial scale. Any scaled-down model used in process characterization or validation should be justified as being representative of the full-scale unit operation (see **Section 8.0**).

Full-scale studies are most frequently used for validation of process consistency and performance and for concurrent lifetime studies of reusable membranes and column resins. Examples of validation studies performed using scaled-down models may include a prospective determination of resin lifetimes, viral clearance, viral inactivation, in-process pool (i.e., process intermediate) hold times, and process solution stability. A discussion should include differences in the manufacturing process between small-scale and full-scale methods, and their potential to affect the results of the studies. The materials used in the scaled-down clearance studies should include chromatographic resins, filter membranes, reagents, and solutions that meet the same specifications as those used in production.

In some cases, scaled-down validation studies must be performed when the full-scale approaches are impractical. For example, validation studies should be scaled down if a process-related contaminant needs to be added (spiked) to the feed for a clearance study. The most common of these spiked studies are used for viral clearance. Introducing study viruses into the manufacturing environment would be totally inappropriate. Spiked studies are also performed when the level of a process-related contaminant is too low to be measured at intermediate points in the process. The clearance capability of the entire downstream process can be evaluated by scaled-down studies where the contaminant is added to the process stream at a sufficiently high amount so that its removal can be determined. That the addition of the spike does not adversely affect the performance of the processing operation should be demonstrated. The limit of detection of the relevant assay should be considered when designing these studies. Results of these spiking experiments can be useful to justify excursions during routine production.

Process intermediate stability studies can be done at either full-scale or in a scaled-down system. The advantage of using a scaled-down system is the lack of impact on the full-scale operations when holding the intermediate for the full hold time. During process intermediate stability studies, the control of bioburden and endotoxin levels should be considered to ensure that the study results are not compromised by adverse levels of microbial activity. Full-scale studies are required for demonstration of microbial control.

The process validation program may include study protocols on the following aspects, as appropriate:

- Cell bank and cell line stability
- Viral clearance (where appropriate)
- Impurity clearance
- Process intermediate stability
- Process solution stability
- Drug substance fill, freeze, thaw, and storage
- Mixing studies (product and process solutions)
- Chromatography resin and reusable filter membrane lifetime validation
- Container closure integrity for drug substance
- Leachable and extractable qualification

The scope and scale of these studies should be based on a rational risk assessment with respect to product safety and efficacy considering the process characterization and pilot data.

4.2.2.1 Validation of Microbial Control for Processing and Hold Steps

4.2.2.1.1 Low Bioburden Processing

The manufacture of most protein drug substance is performed under controlled conditions to minimize contamination of the process stream with bioburden and microbial contaminants such as endotoxins. This may be accomplished by using closed systems, bioburden reduction steps such as filtration, controls of equipment cleanliness and bioburden, raw material controls, and in-process testing to assure acceptable levels of bioburden are maintained. Hold steps provide a potential source of bioburden risk since growth of any present bacteria or fungi could occur. A comprehensive microbial control strategy for the facility and the process should be included that is part of the overall contamination control strategy. One facet of this strategy is validation of bioburden control at various points in the process ***(56)***. A risk assessment should be performed to identify potential areas of contamination and the mitigating controls needed to assure acceptable microbial quality of the process stream and drug substance. The following are some areas that should be addressed during the risk assessment to determine the level of testing during PPQ:

- Raw materials bioburden and endotoxin levels
- Solution and buffer bioburden or endotoxin levels and preparation procedures for the solutions
- Solutions and buffer hold times with respect to microbial quality and growth promotion capability
- Resin storage, use, and lifetime
- Equipment cleaning and sanitization (including equipment clean hold times)
- Assessment of steps where microbial growth is a risk (e.g., room temperature steps with growth-supporting solutions and conditions, tangential flow filtration steps where filters cannot be sterilized, steps with a risk of biofilm formation)
- Bioburden reduction steps (e.g., bactericidal buffers, 0.2-micron filtration)
- In-process bioburden and endotoxin testing and limits, including testing at the end of the allowable hold time in the manufacturing equipment
- Environmental conditions (e.g., room classifications)

Although most biopharmaceutical processes are not conducted as aseptic processes, documentation of microbiological control during PPQ is expected ***(56)***. Control and monitoring of bioburden, which includes bacterial and fungal contaminants that may be present in the process stream, is expected throughout the process. Process validation studies demonstrate that sufficient microbiological control of the process can consistently and reproducibly meet bioburden limits. For critical filters (such as those used for final drug substance filtration), bioburden challenge studies should be conducted.

Process validation studies should include protocols for assessing all in-process intermediate hold times as part of the PPQ (see **Section 4.2.2.4**). An acceptable approach has been to determine the chemical stability (e.g., deamidation, oxidation, or formation of aggregates) of the intermediates at small scale. For the microbial portion of the hold time validation, the allowed storage times in the manufacturing equipment used in the process should be built into the timing for the process validation studies. This can be challenging in a facility where lengthening the manufacturing process time can impact the ability to use the facility or equipment for the next lot. In such cases, the manufacturer may be limited to a hold time for which actual full-scale data are available. The number of at-scale lots used to support the hold time validation for microbial quality control should be justified in the PPQ protocol. Validated hold time is limited to the shortest time from the lots used to support the hold time validation.

Resins that are stored for extended hold times should be evaluated for bioburden and endotoxins at the end of the storage period. The ability of the sanitization process to remove bioburden and endotoxins should also be demonstrated, taking into consideration the potential bioburden load on the resin or membrane after the allowed storage period ***(18)***.

Use of 0.2-micron filters for control of bioburden does not mean that it is acceptable to have high bioburden in the process stream prior to the filter, since microbial contaminants can contain proteases and

other impurities, such as endotoxins, that may not be able to be removed. Sampling for bioburden should normally be taken prior to any bioburden reduction step in order to assess the control of the process ***(56)***.

4.2.2.1.2 Aseptic Processes

Few protein processes include aseptic processing steps, however, there may be a need for some product unit operations to include aseptic processing. The final filling of the drug substance sometimes needs to be performed under aseptic processing conditions. Where this is necessary, additional elements should be considered. Generally, this is done by performing aseptic process simulations with a nutrient-growth medium being substituted for the process stream and subsequent incubation of the media to observe it for growth. The guidelines and requirements for aseptic process simulations are outlined in various regulatory guidance and regulations and will not be detailed here ***(57–59)***. The process validation master plan should include a section that addresses any aseptic processing unit operations. In addition, any use of closed systems that assure the adequacy of the maintenance of sterility (or purity of cultures) should also be validated.

4.2.2.2 Impurity Clearance

Impurity clearance is evaluated through laboratory-scale studies and testing of in-process pools from PPQ lots with demonstration of impurity removals at manufacturing scale during PPQ. Impurities that have the potential to adversely affect product safety or intended biological activity should be consistently demonstrated as being reduced to sufficiently low levels.

An initial scientific assessment should be conducted to identify and select the impurities that require study. Representative impurities are selected based on potential safety concerns or as representative of a class of impurities. The risk assessment should consider biological activity, toxicity, quantity, and proximity of the impurity to the drug substance (i.e., where in the process the impurity is found). The capacity of each step to remove the relevant process impurity should also be considered. Acceptable safety margins should be determined on a case-by-case basis.

Impurities may be divided into two categories: process-related impurities and product-related impurities ***(16)***.

Process-related impurities may be further categorized into:
- **Cell substrate-derived –** e.g., HCP, DNA, retroviral-like particles, endotoxins for Gram-negative fermentations
- **Cell culture-derived –** e.g., inducers, antibiotics, serum components, media components
- **Downstream-derived –** e.g., enzymes, processing reagents, inorganic salts, solvents, leachables, ligands (protein A, Cu^{2+})

Product-related impurities include:
- **Truncated forms**
- **Modified forms –** Deamidated, isomerized, mismatched, disulfide-linked, oxidized, or altered conjugated forms
- **Aggregates**

Clearance studies may be used to eliminate the need for establishing routine lot-to-lot testing (e.g., lot-release specifications) for these impurities.

Removal of adventitious agents should also be evaluated. Types of process contaminants may include:
- Endotoxin
- Bioburden
- Virus

Evaluation of impurity clearance results should include a comparison of impurity levels to targets.

During PPQ, routine bioburden monitoring should be included in the PPQ protocols. This information can then be tied to the outputs in the validation study, ensuring that levels of bioburden do not negatively affect product quality. Comprehensive monitoring of in-process bioburden during PPQ should be sufficient to validate microbial control. Steps specifically claimed to achieve sterility of the product stream should be validated. Evaluation of the impact of bioburden on the endotoxin content of the process stream should also be considered during PPQ. Equipment (e.g., tanks, columns) cleaning and sanitization steps should be validated to remove bioburden to acceptable levels.

4.2.2.3 Viral Clearance

Due to the source of some biological raw and starting materials used in the manufacturing of drug substance (i.e., animal cells, human cells, and/or serum additives), the possibility that the cells and/or components used in cell culture could be contaminated with animal or human viruses is inherent. Some manufacturing processes, such as mammalian cell culture-based, must be designed to achieve removal or inactivation of viruses that may be present (endogenous to a cell line) and to remove or inactivate potentially nondetectable adventitious viral contaminants (exogenous). Processes and facilities should also minimize potential introduction of adventitious agents during the process.

While a brief overview of viral clearance will be presented here, public health and regulatory bodies provide ample guidance on expectations for the evaluation of viral removal or inactivation (viral clearance, regardless of the mechanism) in these processes. The ICH *Quality Guideline Q5A: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin* provides information on conducting viral clearance validation studies ***(33)***. The FDA *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use* also provides information on viral clearance expectations ***(60)***.

In biopharmaceutical processes, typically two or more robust orthogonal viral clearance steps are expected to be validated where viral contamination may be a concern ***(61)***. Steps that typically have the capability to clear viruses include low pH, solvent/detergent treatment (for enveloped viruses), heat, filtration, affinity, and ion-exchange chromatography. Studies are normally conducted by "spiking" the process stream of a unit operation with known amounts of model viruses in a scaled-down model of the step. The removal or inactivation is then demonstrated by testing the processed intermediate for the virus after the unit operation has been performed. Some consideration should be made for end of-resin lifetime testing of viral clearance ***(60, 62)***.

The timing of viral clearance evaluation depends on the source of the cell line, knowledge of viruses in the cell line, and the disease that will be studied in the first clinical trials. For processes that include at least two orthogonal robust viral clearance steps, evaluating the viral clearance is still expected. There are other considerations, however, for products intended for use in serious or life-threatening conditions that have no existing treatment available; in such cases, the use of procedures similar to those previously validated may be considered ***(33)***. The number and types of viruses used will also vary based on these factors. Virus clearance studies may be required by the regulatory agency prior to Phase 1 trials. Consulting the relevant regulatory agency is recommended since some EU countries may not accept this approach ***(63)***. Viral clearance studies for a full panel of model viruses are expected for the support of product registration filings and, in some circumstances, may also be needed prior to pivotal or Phase 3 trials.

Viruses used in clearance studies should be selected based on the history of the cell line, the type of cell line, and the exposure to media and additives that may potentially contain viruses. Viruses relevant to the cell line (such as retroviruses for murine cell lines) as well as model viruses can be selected. The ICH Q5A(R1) guidance should be consulted for additional information on the selection of model viruses. Viruses that can be grown in high enough titers to demonstrate significant reduction should also be considered. The rationale for the selection of viruses should be articulated in the viral clearance

validation protocol. Viruses should never be brought into the manufacturing facilities.

Scaled-down models used for virus clearance studies must be reasonable scientific approximations representative of the full-scale production operations. Some experiments require adjusting parameters to accommodate the bulk of the virus added; the amount of virus might interfere with column-back pressure, for example. The process equivalence should be demonstrated by analyzing the process stream in the model system for relevant attributes. Notwithstanding these adjustments, chromatography model systems should have the same linear velocities, resin type and size, product mass loading, and relative buffer and load volumes as the production system. Virus filtration model systems should have equivalent linear velocities or filtration pressures, product-feed concentration, ratio of feed volume to filter area or flow decay, and operating temperature as the production system ***(64)***. For validation of low-pH hold viral inactivation steps, the pH, temperature, and protein concentration should be similar to the production scale, and the time course of inactivation should be evaluated. Inactivation by low pH should explore the upper end of the acceptable pH range and the minimum exposure time.

Parameters for other steps, such as solvent/detergent treatments, should be equivalent to those used for full-scale operation and should include evaluation of the time course of inactivation. Acceptance criteria for viral clearance validation studies should include removal or inactivation based on a logarithmic scale. For endogenous viruses (e.g., retroviruses), clearance and inactivation beyond the maximum level that could be present in the process must be demonstrated ***(60)***.

4.2.2.4 Intermediate Stability

The biochemical stability of process intermediates needs to be validated; this can be accomplished directly at manufacturing-scale in combination with lab-scale validation studies ***(65)***. While the manufacturing-scale approach may conflict with the manufacturing schedule, lab-scale studies with sufficient scientific rationale, including considerations of material of construction, material-to-container ratios, and material-to-air ratios, can provide relevant data for establishing intermediate hold times from the standpoint of chemical stability. Implementing strategies that minimize the schedule impact and incorporate lab-scale studies can also include holding portions of the intermediate in the manufacturing tanks and testing them over a period of time while the rest of the batch is forward-processed. This approach would minimize the impact of the hold studies on material availability and scheduling. It has been suggested that testing hold times be verified in excess of those anticipated to provide a safety margin. The intermediate stability validation protocol should specify the rationale around the approach chosen, and studies should be performed under the same conditions as will be used for the process.

Cumulative worst-case hold times represent the sum of maximum unit operation hold times. Under normal manufacturing conditions, it is unlikely that all worst-case conditions would occur at the same time and does not require validation. Provided the intermediate is stable and allows meaningful analyses, independent studies of individual steps are likely to be sufficient and cumulative studies are not considered necessary. Manufacturing of biological drug substance intermediates is not claimed to be a sterile operation and therefore cannot be validated as such. Yet, excessive bioburden could damage product quality or introduce unknown contaminants, so limits must be set. Consequently, documentation of process intermediate bioburden and endotoxin control at manufacturing-scale should be included in the PPQ, which will require routine monitoring of process intermediate bioburden and endotoxin levels during manufacturing operations (see **Section 4.2.2.1)**.

4.2.2.5 Process Solution Stability

The manufacture of drug substances typically requires numerous process solutions that do not contain cells or the molecule of interest, including cell culture media, dilution buffers, viral inactivation solutions, and chromatography buffers. All buffers and solutions should be assessed with a plan to demonstrate stability. This assessment may include grouping buffers into "families" with similar properties and performing chemical stability on worst-case buffers. A science-based rationale should be given in

the protocol as to why a specific buffer is chosen to represent a family. An assessment of the capability of the buffer or solution to support microbial growth should be performed and, if appropriate, bioburden studies should be performed as well.

The most straightforward way to validate the stability of solutions and buffers is to conduct hold time studies during which the solutions are held over the maximum duration in full-scale tanks. The validation protocol should address bioburden, endotoxin, and chemical stability. If stored in a single-use system (SUS), the issue of extractables and leachables (E&L) also should be addressed (see **Section 4.2.2.9**). Actual buffer-release criteria during manufacturing are helpful in establishing parameters; that could include the measurement of bioburden, endotoxin, pH, conductivity, and/or buffer-specific assays. This approach will provide a robust validation, but it requires access to manufacturing tanks over a significant amount of time, as the requirements for buffer hold times may be several weeks. Investigation of chemical stability, such as for buffers or solutions like phosphate-buffered saline or sodium chloride, may not be necessary if they are appropriately justified by manufacturing experience or literature.

If access to the required manufacturing tanks is not feasible prior to PPQ, hold time studies of the buffers can be validated concurrently at manufacturing scale. This can be accomplished by preparing more solution than required for manufacturing, testing the solution for release, using it in manufacturing for as long as possible, testing the remainder of the solution again, holding it for as long as possible until the tank is needed in manufacturing again, and testing it before disposal.

If the buffers will be held in sterile tanks or sterile single-use bags, validation of the sterile hold duration and chemical stability can be separated. With this strategy, the capability of the tank or SUS, including associated equipment, to hold a solution under sterile conditions is documented during the initial qualification of the tank or bag system. To document worst-case scenarios, process simulation studies using tryptic soy broth media may be conducted for sterility assurance verification. Once the capability of the equipment to hold solutions under sterile conditions in the actual manufacturing area has been documented, the chemical stability of the solution can be addressed at small scale. The small-scale container should be made of identical material of construction as the full-scale container and should represent a worst-case scenario with respect to the solution-tank or solution-bag surface interface (i.e., surface/volume ratio) and the air-liquid interface. Justification for the scale-down model should be included in the protocol.

4.2.2.6 Mixing Studies

Biotechnology processes typically may involve pooling of different liquids or dissolution of powders into solution. The study approach is similar for both pooling and dissolution and involves similar decisions. Typical factors to consider are the effect on the product from mixing time, the rotations per minute (RPM) of the mixing equipment, the mass transfer coefficient (k_La), the power per unit volume (PPV), and the temperature. The relative importance of these factors depends on the materials being mixed. Values for temperature and RPM usually require a range around a set point, and mixing time is typically expressed as a minimum value (e.g., mix for at least 15 minutes at a set point of 12 RPM (10–14 RPM) at 25 °C (20–27 °C) in specified equipment). Establishing maximum mixing times, where appropriate (e.g., when shear, oxidation, and product degradation are considerations), should also be considered. A grouping approach can be applied to mixing studies (e.g., by using buffers with the highest concentration or less soluble compounds). If appropriately justified, these studies can be representative for all other buffers.

Mixing studies typically require sampling from the mixing vessel at specified time intervals (and possibly at levels in the equipment), assaying the samples, and then plotting the results against mixing time. The appropriate parameter for the solution should be identified and measured (e.g., density, conductivity, protein aggregation, stability or, in the case of powders, clarity of solution). If two solu-

tions of different densities are being mixed, they might be sampled at different locations in the vessel. The minimum mixing time required is the point at which homogenous results are obtained and assay results vary only within the assay variability. Visual inspection for complete dissolution of powders may also be appropriate. The mixing study protocol should include the rationale for the parameters selected and the attribute acceptance criteria.

4.2.2.7 Freeze-Thaw Studies

Freeze-thaw validation studies for the drug substance manufacturing process should include the steps necessary to store intermediates and the purified bulk material (drug substance) prior to formulation and filling of the drug product. These operations follow the final step, in which the protein is purified or has its buffer and concentration adjusted. If intermediates or drug substance are stored frozen, studies should encompass the freezing and subsequent thawing steps and should minimally mimic the number of freeze-thaw cycles that are anticipated. Performing an additional cycle beyond the number expected may also be prudent for added assurance of stability. Generally, drug substance sterility is not a requirement. If sterility is not being claimed, then an appropriately low bioburden specification should be set. If sterility is claimed at this step in the process, then the sterilizing filter must be validated. This is typically performed by contracting with the filter manufacturer, a process that is fully described in PDA *Technical Report No. 26 (Revised 2008): Sterilizing Filtration of Liquids* ***(66)***.

Analogous to the approach for other process steps, the operations necessary for storing the drug substance should be characterized in the laboratory using a scaled-down model prior to preparing freeze-thaw study protocols. Critical parameters, if any, should be identified and monitored during the conformance lots. For frozen storage, the temperature, concentration, and pH gradients during freezing and thawing may be of particular concern. Models that are appropriate for examining these effects in the laboratory have been described in literature ***(67, 68)***.

Typical operating parameters for freeze-thaw processes may include the following:

- Product concentration
- Buffer concentration
- Sterilizing-grade filter integrity (even if sterility is not claimed)
- Freezer temperature (for frozen storage)
- Freezing rate (for frozen storage)
- Thawing temperature (for frozen storage)
- Thawing rate (for frozen storage)
- Light exposure during storage
- Container-closure type and size

Bulk container acceptance criteria in the protocol may include the following:

- Bioburden after bulk container filling (prior to freezing)
- Bioburden after bulk container thawing (prior to transfer from container)
- Product concentration
- Product stream conductivity and osmolality
- Product stream pH
- Product quality

Drug substance storage is generally addressed as part of the formal drug substance stability program. The ICH has published specific guidelines for stability programs in *Quality Guideline Q5C: Stability Testing of Biotechnological/Biological Products* ***(65)***. Container product-contact material is tested as part of the stability program, and there may be compatibility studies conducted prior to stability testing. For example, storage CCS, hydrophilic filters, and other bulk filling materials made of polymeric (plastic) wetted materials should be tested for E&L. Any observed leachables should be identified and assessed for safety and product compatibility. Storage of product for stability testing should be

representative of the actual storage conditions. Storage CCS should also be validated for seal integrity.

4.2.2.8 Resin and Membrane Reuse Studies

The ability of chromatography resins and reusable membranes to function consistently over their intended lifetime (i.e., lifecycle) should be demonstrated as part of process validation ***(18)***. Each of these materials has specific, process-defined functions that need to be evaluated to demonstrate that the materials and their related procedures are suitable for the intended purpose. This can be accomplished through various combinations of scaled-down, full-scale, prospective, and concurrent validation studies, as well as through emerging technologies using PAT.

Chromatography resins and reusable filter membranes are initially evaluated in scaled-down characterization studies to determine operating parameters and performance attributes. In addition, the cleaning procedure for both resins and membranes should be evaluated, not only for effectiveness of the cleaning (including elimination of carryover of impurities), but for effect on the lifetime of the resin ***(69)***. These findings are then applied to evaluation of the resin's performance over time to determine how long they can be used in the manufacturing process. Lifetime evaluation should consider the number of cycles. Small-scale characterization studies are typically performed to establish a recommended lifetime (i.e., maximum number of use cycles). Generally, characterization studies might entail the evaluation of multiple lots, with one lot of resin being sufficient for lifetime studies.

Once the scaled-down model system has been demonstrated to approximate performance of the full-scale process, the small-scale study may be used to prospectively establish the number of cycles for chromatography resins and reusable membranes. The advantage of the scaled-down systems is that they can be completed independent of manufacturing operations. These studies provide justification for establishing initial lifetime and baseline performance expectations. In addition, the scaled-down studies can provide a model for "aged resin" that can be used for evaluation of end-of-use viral clearance capacity.

Concurrent full-scale studies are performed to confirm scaled-down lifetime studies and continue past PPQ runs. A concurrent lifetime validation protocol follows a set of predefined parameters during the course of manufacturing. This may consist of measurements such as height equivalent to a theoretical plate, water flux, yield, or removal of a process-related impurity.

Evaluation of performance parameters is dependent on the functional purpose of the unit operation, regardless of the scale or time-course of the lifetime studies (e.g., reusable membranes might be monitored for integrity, water and process flux, and product yield). Monitoring frequency of the parameter measurements should be determined based on the specific operation and timing of data availability. Sampling should be done on a schedule that will allow trends to be identified before ranges are exceeded.

Storage times and conditions required to maintain performance characteristics of the resin or membrane for the intended lifetime should also be demonstrated. Consideration should be given to life span as well as the number of cycles ***(70, 71)***. Testing of the storage solution for bacteriostasis and fungistasis may be part of these studies.

Solutions used for cleaning and sanitization of column resins and filtration membranes should be chosen based on the supplier's recommendations, supplier's compatibility data, and/or internal studies. Cleaning and sanitization solutions should be chosen based on their ability to remove process soils, inactivate microbial contaminants, and reduce levels of endotoxin. Routine use of these solutions should be included in the column resin and membrane lifetime studies. Cleaning validation studies (confirmation of removal of process soils to acceptable residual levels) are often performed parallel to lifetime studies.

4.2.2.9 Extractables and Leachables

All product-contact surfaces used in a manufacturing process present the possibility of leaching extractables into the process stream. While modern materials of construction are typically very low in extractables, some consideration should be given to the nature and potential amount of extractables. This can be done using a risk assessment tool that weighs such characteristics as surface area, contact time, temperature, pretreatment (gamma radiation or steam sterilization), and proximity of the item to the final product ***(29)***. Compilation of the manufacturer's data and published literature, along with a resulting low-risk priority score, may be sufficient to document the relative safety of the material used at a given step in the process. Chemical compatibility is part of that risk assessment but, ideally, compatibility considerations were addressed and documented during the design and development phases of the process.

With the broader use and adoption of single-use components in the GMP biomanufacturing environment, consideration of E&L has been pushed upstream in the process from where it was once well known and refined in the primary packaging arena. This complex issue goes far beyond the general concern for patient safety; it may be the root cause for poor cell growth, protein instability, protein aggregation, particulate formation, and a reduced level of key ingredients ***(72-74)***.

A number of industry organizations have published guidances or recommendations about the E&L issue, as well as individuals and companies ***(75-78)***. The Biophorum Operations Group presents a broad approach that covers risk assessment methodology, supplier extractable data review, and the final leachable study requirement from component selection to qualification ***(79, 80)***. Despite the multitude of efforts, none of these works fully addresses the complexities of E&L in totality. Nevertheless, they provide a foundation upon which a company can develop its comprehensive strategy to evaluate and mitigate such risks in the biomanufacturing environment.

Evaluations of E&L are generally restricted to process fluid contact with single-use components during the manufacturing process. Full E&L evaluation can be separated into three interlinked topics: risk assessment, vendor-supplied extractable data evaluation, and process-specific E&L data.

4.2.2.9.1 Risk Assessment

The general principle of the risk assessment is focused on the amount of leachables from the material pertaining to such key characteristics as organic content, pH level, contact duration, contact time, contact temperature, material characteristic, use of the material in relationship to the entire process, and surface area-to-volume ratio. Regardless of the design of the model, the main purpose of the risk assessment is to provide a guide as to where companies need to focus their E&L assessment and qualification efforts. Most of the published risk assessment models to-date have been geared toward leachable risk associated with the use of the material regarding patient safety, however, not necessarily assessing the risk to cell growth, protein instability, protein aggregation, particulate formation, or reduction of key ingredients.

4.2.2.9.2 Vendor-Supplied Extractable Data Evaluation

Generally, available supplier data is gathered during the selection phase to ensure such data can be used to prescreen any potential leachable risk to patient safety, cell growth, protein instability, protein aggregation, particulate formation, and potential reduction of key ingredients. The full extent of this prescreening activity greatly depends on the user's knowledge of their particular process. The user can elect to perform specific studies in the selection phase to assess the other risks related to manufacturability and product qualities beyond patient safety ***(79)***.

4.2.2.9.3 Process-Specific E&L Studies

Full E&L evaluation is necessary for license applications depending on the level of risk the risk assessment tool reveals.

Gathering all the relevant information about the component(s) before this E&L evaluation would be advantageous in order to complete this activity without lengthy delay due to missing information. Such information can include, but is not limited to, material of construction, component part number and trade name, chemical compatibility, pretreatment, wetted surface area (cm^2), and safety and biocompatibility ***(81)***</author></authors></contributors><titles><title>Guideline for Use of Single-Use User Requirements (SUUR. Some of this information may require communications with sub-suppliers and confidential disclosure or non-disclosure agreements.

Evaluation of E&L can be satisfied by leveraging available extractable data that can bracket the intended-use condition of the single-use component in terms of the fluid matrix (e.g., pH and organic solvent content), operating temperature, and contact duration. In circumstances where the available data cannot bracket the intended-use conditions, simulation and/or leachable studies will be necessary.

4.2.3 Validation of Single-Use Systems

SUS are now a generally accepted alternative to traditional stainless steel-based systems in all bioprocess steps, including cell culture and fermentation, harvesting, purification, mixing, storage, freeze/thaw, and fill/finish applications. Detailed information on implementation considerations can be found in TR-66 ***(40)***. The materials used to fabricate SUS are primarily polymeric materials, and their physical and chemical properties can be influenced by many factors, including molecular structure of the SUS construction materials, additives, processing methods, and other conditions such as heat, light, oxygen, chemical contact, and sterilization. Any material considerations should be addressed during single-use material or consumable qualification. Therefore, validation for SUS and the user's process must be robust enough to tolerate any variations of the SUS manufactured by the suppliers ***(65)***. The purpose of the validation is to demonstrate that the SUS can provide high levels of security and robustness to consistently produce a product meeting its predetermined specification and quality characteristics, and to prove that the SUS is able to maintain structural integrity (e.g., no leaks) with no adverse effects to the product in contact (e.g., E&L). These activities should be defined within the product process validation lifecycle to effectively address all related concerns and their potential impacts to subsequent pharmaceutical product validation approaches.

Unlike traditional multiple-use systems, SUS come with "ready-to-use" features; they are preassembled, sterilized, and supplier-tested based on their intended use. Suppliers often characterize their products, conducting such basic suitability testing as biocompatibility, bioburden, endotoxin, and particulate levels. Sterilization may be performed before material use through gamma irradiation or autoclaving; thus, the associated cleaning validation and autoclave cycle validation efforts no longer need to be performed by pharmaceutical users. Most data on physical and mechanical properties of the systems can also be provided by the SUS suppliers, including tensile, puncture resistance, and gas and water vapor transmission. SUS suppliers are also able to provide basic chemical compatibility data of the contact polymer materials with common reagents, which can serve as good starting points for a user's evaluation of the potential systems and components. Performance qualification is unique to each SUS; the supplier's performance validation guide is often provided to demonstrate whether the item can meet the basic performance expectations in bioprocessing. For example, aseptic connectors may need to be tested for burst pressure, creep rupture, functional performance (such as water flow characterization), soiling testing, and tightness (i.e., resistance to bacterial ingress). Supplier validation data packages may also include other validation studies, such as mixing, storage, shipping, freeze/thaw, and filter validation, based on the component's intended use. In addition, E&L is still one of the primary concerns that prevent SUS from being adopted into some companies' processes as detailed in **Section 4.2.2.9**.

Other SUS product material information may also be provided by item suppliers. The biopharmaceutical company users heavily rely on supplier study data and documentation to meet component function and compliance requirements. Ultimately, however, it is the drug manufacturer's responsibility

to demonstrate process robustness and product safety. Many SUS, especially systems used in critical process steps, must be further validated to demonstrate their suitability for bioprocessing, because the supplier data are often general and may not take the user's process-specific requirements, such as temperature, pressure, concentration, or duration, into consideration. A good example is filter validation data, where the filter manufacturer may provide bacterial challenge information establishing a correlation of integrity test values to bacterial retention under defined conditions, but the user must still validate bacterial retention under worst-case process conditions with the actual formulation. Similarly, prepacked, pre-validated disposable chromatography columns are a recent addition to the single-use category and are available in a range of sizes and resin types to accommodate purification techniques based on the product's physical or chemical properties. Supplier validation usually focuses on physical characteristics of the columns such as pressure difference, flow rate, capacity, and resin lifecycles. The user still must perform full validation on the single-use chromatography systems in order to establish process limits for desired product impurity clearance, elution profile, yield, and purity. For E&L, in general, suppliers can provide extractables evaluation and data on their designed conditions for initial component screening, but the users must evaluate the components and systems under the actual use conditions (e.g., leachables study) that are specific to the product and process.

Overall, SUS must offer similar levels of security and robustness as traditional multiuse systems. A supplier may be able to supply a large amount of SUS validation data, but the user must be aware of the scope and limitation of the data presented. Careful comparison of the available validation data from the suppliers to the data required by the user is needed to make an informed, risk-based decision regarding process-specific validation. If full validation is not needed, a justification of the reduced validation approach should be provided. In addition, due to the complex supply chains of SUS, supplier-initiated changes occur frequently. A change management program should be established to evaluate the impact of any proposed change on the system and its validation status, and a risk assessment for each change should be carefully performed case by case based on the material-specific applications in bioprocessing. Partial or full revalidation studies could be needed when the proposed change might affect product quality or when it might include additional process design and process qualification activities. These situations must be strictly handled through appropriate change management systems.

5.0 Stage 3: Lifecycle Management

5.1 Ongoing Process Verification Approaches

Once a process is validated, the product lifecycle continues with routine ongoing monitoring and evaluation of the process. Ongoing process monitoring provides evidence that the process remains in a state of control and is within the validated state during commercial manufacture. OPV, also known as continued process verification, begins immediately after successful validation with the introduction of an approved process monitoring plan. This plan includes the process inputs (process parameters) and outputs (performance and quality attributes) that define successful operation of the process. Specific in-process controls are put in place to monitor the process. These controls typically include routine sampling and monitoring (e.g., of cell culture titers, bioburden, adventitious agents, endotoxin, chromatograms, in-process impurity levels, and yield) at specific, defined steps of the process. The basis of OPV is often the process control strategy, including drug substance release-testing, defined during validation. At a minimum, the parameters defined as critical need to be routinely monitored as part of the OPV. This may also include aspects related to cold chain management or shipping validation. Further detailed information on these aspects of OPV can be found in various PDA technical reports ***(11, 13, 82, 83)***.

Additional testing and monitoring points (such as growth and metabolite profiles and chromatograms) are commonly part of OPV. Characterization tests are acceptable as part of the OPV plan (especially directly after validation and before enough lots have been completed for reliable statistical analysis) for process understanding or until sufficient data are collected to statistically establish clear limits. If the output data from characterization tests do not merit limits or acceptance criteria, then the tests can be eliminated and reinstituted for comparability exercises as needed. Once the value (or lack thereof) of the test is decided, either the test is dropped, or the limits are set. Routine sampling and monitoring locations should be included in the production documentation (e.g., manufacturing batch records). In-process tests are performed at critical decision-making steps or at steps that serve to confirm consistency of the process ***(16)***. These tests usually have action limits or, in some cases, acceptance criteria. Since the limits and acceptance criteria may be based on data from a limited number of developmental, pilot, and commercial-scale lots, the monitoring points and, where defined, range adequacy must be continually reevaluated as additional data on commercial-scale lots are accumulated. Additional analyses of parameters can provide statistically sound data for process optimization and enhanced control. The OPV plan becomes the statistical process control of the process ***(1, 2, 4, 84)***.

Routine monitoring steps should be included in the production documentation. Trending of the manufacturing data must be done at established intervals (e.g., quarterly for high-production volume products or annually for low-production volume products); trending is also useful in the ongoing evaluation to ensure the process is operating within its validated state. The knowledge gained from continued process trending will improve overall process understanding, allow for confirmation or modification of the approved process control strategy, and provide an early warning when attributes are starting to drift. Statistical analysis and trending of the data should be applied wherever feasible to alert the company to adverse process behavior. Statisticians should be consulted to help design a framework that can be used to analyze the trending data, at a minimum, as different tools are required depending on the normality of the data, the sample size ($n > 30$ is typical for establishing permanent limits), the randomness (autocorrelation), and determination of control limits. Standard control charts, using the moving-range method, and process capability (Cpk/Ppk) are commonly used, have proven useful, and are described in the literature ***(21)***. Processes with Cpk/Ppk ≥ 2.0 are considered highly capable, between 1.0 and 2.0 are marginally capable, and <1.0 are not capable of meeting the specification and process improvements.

5.2 Transfer of Biopharmaceutical Manufacturing Processes

Regulatory requirements for biopharmaceutical products should be considered when manufacturing process technology is transferred. Health authority approval is required before products manufactured at the receiving site can be commercially distributed. The process of technology transfer should consist of planned and controlled actions based on well-defined acceptance criteria. Proper conveyance of a manufacturing process, analytical method, packaging component, or any other step or process along the biopharmaceutical product lifecycle is crucial as process variations can have a significant impact on biological end-product potency and function. Further information and strategies for conducting successful technology transfer can be found in PDA *Technical Report No. 65: Technology Transfer* ***(85)***.

5.2.1 Process Transfer and Adaptation

The process established at the originating site may require technical adaptations and facility fit of the approved process (e.g., tank volume differences, scale-up, different filters, digital control systems) to accommodate the facility, equipment, and provisions of the receiving site. Technical adaptations or process changes made in a site-to-site transfer for facility fit must be described, justified, and supported by a successful process validation program. Technical adaptations must be fully evaluated using risk-based approaches similar to those used for the validation of the sending site, and the process at the receiving site must achieve comparable outputs. Process modifications (i.e., addition, removal, or replacement of one step with another) are typically considered different processes and are usually not allowed within the same marketing authorization; therefore, a separate filing would be required.

5.2.2 Product Comparability and Stability

Comparability between products manufactured at the originating and receiving sites must be demonstrated. Physiochemical and biological characterization data will demonstrate comparable quality between pre- and post-transfer products. Stability data (including stability under accelerated conditions) is used to further support comparability ***(86)***.

In cases where analytical comparability is insufficient or inconclusive, additional nonclinical or clinical data may be required before the health authority will approve manufacturing at a second site.

5.2.3 Multisite Manufacturing of Biopharmaceutical Products

Where products are manufactured at more than one production site, processes at both sites must remain harmonized. Any lifecycle activity or process improvement initiative must be evaluated for implementation at all sites.

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7.0 Appendix 1: Example of Failure Modes and Effects Analysis

As noted in **Section 3.6.1**, the **Table 7.0-1** provides an example of an FMEA and the subsequent RPN assigned for the control strategy design.

Table 7.0-1 Example FMEA: Failure Modes and Effects Analysis

Unit Process	Process Variable	Critical Aspect	Potential Failure Mode	Potential Cause	Potential Effect(s) of Failure to Quality/ Consistency Attribute	Impurities	Contaminants	Regulatory Requirements	Parameter Criticality	Severity	Existing Design / Prevention Controls	Occurrence	Existing Design / Detection Controls	Detection	RPN	Risk Accepted?	Acceptance Justification	Risk Mitigation Action(s)
Working Cell Bank for Vial Thaw	Centrifugation Time	Meeting Setpoint	Does not meet setpoint	1. Human Error 2. Equipment Error (centrifuge)	TBD	TBD	TBD	TBD	TBD	1	1. Equipment Qualification and Calibration 2. Inclusion of Setpoint in MBRs 3. Operator Training	1	1. Operator Verification 2. Batch Record	2	2	N/A	N/A	N/A
50L Single-Use Bioreactor (N-4 and N-3)	Incubator temperature for medium conditioning	Remain within acceptable range	Exceed acceptable range	Equipment Error (single-use bioreactor, incubator)	TBD	TBD	TBD	TBD	TBD	1	1. Equipment Qualification and Calibration 2. Include Range in Batch Record 3. MCS Monitoring	2	1. Batch Record 2. IPC Testing	4	8	Yes	N/A	N/A
Protein A Chromatography	Elution flow rate	Remain within upper acceptable limit	Outside acceptable range	1. Equipment Error 2. Flow Fluctuation during pump initiation	TBD	TBD	TBD	TBD	CPP	3	1. MBR and unicorn methods are GMP reviewed and approved 2. Flow meters are calibrated and PM'ed	3	1. Operators monitor method via graphical user interface 2. Flow Alarms	2	18	No	N/A	1. Lower bed height 2. Decrease elution flow rate
UFDF1	Hold Time	Remain within acceptable range	Outside acceptable range	1. Human Error	TBD	TBD	TBD	TBD	CPP	3	The UFDF1 pool expiry date is calculated and verified in MBR	4	2nd-Person Verification	3	36	No	N/A	Option of chilling UFDF1 pool can be enabled to extend hold time beyond 24 hours
Formulation, Filtration and Fill	Filling - Fill weight / volume	Remain within acceptable range	Outside acceptable range	1. Operator Error 2. Scale Error 3. Hold up Volume not considered for last bag	TBD	TBD	TBD	TBD	Non-CPP	2	1. Product net weight is verified in MBR 2. Print-out of weight is attached	4	MBR is reviewed prior to batch release	3	24	No	N/A	Hold up volume in the line to be considered before calculating the total number of bags to be filled

8.0 Appendix 2: Example of Scale-Down Model Qualification

Scale-down model verification requires comparing multiple runs at small scale to full-scale batches using a combination of proportional and identical input process parameter values (see **Table 8.0-1**). Ideally, the verification should include data from runs spanning the entire operating range; however, this is not often practical. The initial verification should, at least, be completed using multiple runs at center point.

The verification of scale-down models should include a predefined number of runs at each scale and with prospectively defined acceptance criteria (e.g., step yield, final bioreactor titer, and product quality attributes). Scale-down model verification should be similar to the full-scale process validation in design. The number of replicates (*n*) should be aligned with the overall risk of failure; *n* typically equals three (*n*=3). A scale-down verification example is presented in **Table 8.0-2**.

Table 8.0-1 Scale-Down Variables

Unit Operation	Constant Variables	Proportional Variables
Bioreactor/Fermenters	P/V; k_La; T	rpm; D_i; V; D_t; V_s
Chromatography Columns	L; protein load (g/L resin); VF; t_R; wash volumes (in CV_s)	ID; F; CV
Tangential Flow Filtration	Protein load (g/A); Flux; TMP	A; F
Depth Filtration	Protein load (g/A); max inlet pressure	A
Stability	T; material of construction; light exposure	V

P/V = power per unit volume, k_La = liquid-phase mass transfer coefficient, T = temperature, rpm = revolutions per minute, Di = impeller diameter, V = volume, D_t = tank diameter, Vs = superficial gas velocity, L = bed height, VF = linear fluid velocity, t_R = residence time, ID = column inner diameter, F = volumetric flow rate, CV = column volume, TMP = transmembrane pressure, A = membrane area

Table 8.0-2 Chromatography Column Scale-Down Verification Example

Scale	Criteria	Yield (%)	HCP (ppm)	DNA (ppm)	Eluate Pool Volume (CV)	Criteria Met (Yes/No)
		≤ 10% difference between scales	≤ 50	≤ 50	≤ 10% difference between scales	
Small-Scale	Run 1	88	10	23	1.7	Yes
	Run 2	92	13	28	1.5	
	Run 3	90	15	32	1.6	
	Average	90	13	28	1.6	
Large-Scale	Run 1	87	9	25	1.8	
Historical Mean		88	10	26	1.7	

HCP = host-cell protein; CV = column volume

9.0 Appendix 3: Unit Operations Used in Biopharma Processes

This summary of typical unit operations is accompanied by example process parameters (inputs) and performance indicators (outputs) that could be considered for evaluation during Stage 2 of process validation, based on the primary function of that unit operation. Ultimately, the knowledge gathered through process design, risk assessments, and impact of critical quality attributes (CQAs), as determined in Stage 1, will drive the most appropriate process parameters and performance indicators to be evaluated in a given process performance qualification (PPQ) exercise.

9.1 Expression Construct and Cell-Line Development and Characterization

Biotechnology drug substances are derived from cellular sources that have the potential to influence the consistency of the manufacturing process and the quality of the drug substance. For example, if the cell line or genetic construct is unstable, variability in the amounts of impurities (e.g., host proteins, nucleic acids, and other biological constituents) may result. The purification process may not have the capability to remove the various amounts of impurities, although the process is required to remove or inactivate adventitious agents (e.g., mammalian virus). Therefore, adequate characterization of the starting cellular materials is necessary to understand and control the outcome of the manufacturing process. The ICH guidance available on how to approach the necessary characterization—*ICH Q5B: Quality of Biotechnological Products: Analysis of the Expression Construct in Cells Used for Production of R-DNA Derived Protein Products* and *ICH Q5D: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products*—also summarize current regulatory thinking ***(34, 87)***.

Typically, production cell lines are qualified during generation of the master cell bank (MCB) and working cell bank (WCB) to assure product safety during clinical development; changes made after MCB qualification can often slow the development timeline. Studies must demonstrate the effectiveness of the manufacturing process in eliminating adventitious agents and controlling cell-derived impurities using small-scale models that reflect the amplification of the MCB or WCB cells. The studies quantify risk and demonstrate that an adventitious virus introduced into the cell cultures during manufacture can be adequately removed or inactivated. Demonstrating that excess capacity to eliminate adventitious agents is built into the process to assure safety of the drug substance is very important.

Assessment of the cell line, with respect to consistent production of the intended drug substance, depends on the nature of the cell substrate, the cultivation methods, and the target protein. For recombinant DNA expression constructs, consistency of the coding sequence of the expression construct should be demonstrated in cells cultivated to the limit of in vitro cell age for production use, either by nucleic acid testing or product analysis. The limit should be based on data derived from cells expanded under representative conditions to the proposed in vitro cell age or beyond.

9.2 Vial Thaw and Cell Expansion (Seed Train)

Primary function: Generate sufficient viable cell mass to inoculate the subsequent expansion stage at the required viable cell density. This step usually begins with thawing the cell line and expansions in a series of increasing volumes of growth stages (**Table 9.2-1**). Product is not produced at this stage, but process consistency in the biomass is important.

9.3 Production Bioreactor (Fermentation/Cell Culture)

Primary function: Generate protein product with consistent desired product quality. At this stage, the desired product is produced in the cells or secreted into the culture medium (**Table 9.3-1**). Inducers or selective pressure may be applied, and supplemental growth additives may be added to the culture. Different systems, such as single-use bioreactors, perfusions cultures, or batch fermentation in stainless steel bioreactors, may be used.

Table 9.2-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Vial Thaw	Temperature Duration	Viable cell density Viability
Scale-Up (e.g., flasks, wave bags, seed bioreactors)	Temperature Duration Incubation % CO_2 Agitation rate Target seed density	Viable cell density Viability Purity

Table 9.3-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Production Bioreactor	Temperature pH Duration Air/gas sparging rate Agitation rate Feed composition, concentration, and rate Target seed density	Viable cell density Viability Specific growth rate Dissolved O_2 Product yield Purity Adventitious agents Mycoplasma

9.4 Cell Culture

Primary function: Consistently generate protein product with the desired product quality. Cell culture and fermentation can loosely be categorized as batch, fed-batch, or perfusion (continuous). Perfusion culture involves simultaneously adding and removing equivalent volumes of media while retaining the cells in the bioreactor to achieve a pseudo-steady state (**Table 9.4-1**). Fresh feed material (e.g., sugars, nutrients) are continuously pumped into the bioreactor while spent media, impurities, and the target molecule (for secreted systems) flow from the bioreactor to either a holding vessel or the next unit operation for further processing. The cells are retained in the bioreactor using membranes operated in tangential flow mode. The constant replacement of media provides a steady source of fresh nutrients for the cells and a constant removal of cell waste-products. The media volume requirements

Table 9.4-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Bioreactor	Temperature pH Air/gas sparging rate Agitation rate Feed composition, concentration, and rate Target seed density Perfusion rate and duration (perfusion system only) Shear rate Membrane size	Viable cell density Viability Specific growth rate Dissolved O_2 Product yield Purity Adventitious agents Mycoplasma

for perfusion are far greater than for fed-batch and often exceed one reactor volume per day. The large volume of media necessitates additional media preparation, media storage, and disposal of spent media. Batch culture entails adding all components at the beginning of the manufacturing process. Fed-batch relies on removal of media components following each growth and harvest cycle.

Most bioreactors, both stainless steel and disposables, are capable of operating in perfusion mode as long as a cell retention mechanism is added. Cell retention can, for example, be achieved using a tangential flow step with either a flat sheet or a hollow fiber membrane, where the cells are retained, and the cell culture fluid passes through the membrane.

Perfusion can be implemented at many process stages: high-density, large-volume cell banking; cell expansion up to the *n*–1 step; and production bioreactors. Perfusion steps in the cell expansion steps are used to deliver continuous high-density inoculums to the subsequent step, often as a part of an intensified process. Production bioreactor steps using perfusion can have a two- or three-month duration, or they may be only a two- or three-day jumpstart to a standard fed-batch process.

9.5 Harvest

Primary function: Separate product from cell culture matrix in preparation of purification. In this stage, various separation techniques are applied to separate the biomass from the culture medium (**Table 9.5-1**). In some cases (e.g., *E. Coli* inclusion body process), the cell mass will be collected for forward-processing and, in others, the supernatant will contain the desired product.

9.6 Capture Chromatography

Primary function: Capture product and reduce process-related impurities and cell-culture media components. This usually constitutes the first step of the downstream process, where the desired product is enriched through a specific resin designed to capture the desired product, while impurities flow through and are reduced (**Table 9.6-1**).

9.7 Viral Inactivation

Primary function: Inactivate enveloped viruses without negative impact to product (**Table 9.7-1**).

9.8 Polishing Chromatography

Primary function: Polish purification to reduce process- and product-related impurities (**Table 9.8-1**).

Table 9.5-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Acid Precipitation	pH Temperature Time	Packed cell volume
Centrifugation	Feed flow rate Bowl speed Discharge interval	Product yield
Filtration	Flow rate Filter loading	Product yield

Table 9.6-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Load	Load factor Temperature Flow rate (based on linear velocity)	N/A
Wash	Buffer composition and concentration pH Volume Flow rate (based on linear velocity)	N/A
Elution	Buffer composition and concentration pH Volume Flow rate (based on linear velocity) Start and end collection criterion	Product yield Peak shape Purity

Table 9.7-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Examples – solvent detergent, low pH, inactivating chemical(s)	pH Time Temperature Mixing	Product yield Purity Note: viral reduction demonstrated through separate small-scale studies
Low pH Viral Inactivation	pH	The pH of the viral inactivation pool is validated in small-scale studies. Failure to reach the validated pH range may result in incomplete inactivation of enveloped virus.
Low pH Viral Inactivation	Incubation time	The pool is held under low pH conditions for a specified time, as validated during viral clearance validation. Failure to hold the pool for the validated duration may result in incomplete inactivation of enveloped virus.
Viral Filtration	Differential pressure	Manufacturer's recommendation for maximum differential pressure on viral removal filters must be observed to prevent potential viral breakthrough.

Table 9.8-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators	Performance Indicators
Load	Load factor Temperature	Flow rate (based on linear velocity)	N/A
Wash	Buffer composition and concentration pH	Volume Flow rate (based on linear velocity)	N/A
Elution	Buffer type and concentration Gradient slope pH	Volume Flow rate (based on linear velocity) Start and end collection criterion	Product yield Peak shape Purity

9.9 Ultrafiltration and Diafiltration

Primary function: Exchange product into formulation buffer and target concentration (**Table 9.9-1**).

9.10 Drug Substance Filling

Primary function: Fill storage containers (e.g., carboys, bags) for intermediate storage prior to drug product processing (**Table 9.10-1**).

9.11 Single-use Bioreactor Cell Culture

Traditional fermentation and cell culture processing used glass bioreactors for lab- and small pilot-scale testing and used a stainless-steel bioreactor for larger applications. Plastic, disposable, single-use bioreactors are increasingly being used in place of stainless-steel bioreactors for clinical and commercial processes. These single-use bioreactors (SUBs) use a bag that is supported by a rigid structure that also provides temperature control. The bag is used once and then discarded, while the rigid holder, which is not product-contact, is reused multiple times. Single-use containers consist of multiple layers; each layer provides mechanical stability, gas permeability, or inert gas for product-contact. SUBs use either rocking or traditional stirring technologies along with common probes to measure pH, temperature, dissolved oxygen, and other factors. In contrast to stainless-steel bioreactors, most disposable containers need to be assembled prior to use. The qualification of SUBs is very similar to the qualification of permanent bioreactors with one exception: SUBs do not need confirmation of cleaning and sanitization.

9.12 Reactions

Primary function: Reduce process- and product-related impurities by chemical or enzymatic reaction (**Table 9.12-1**).

9.13 Conjugation (e.g., Drug, Vaccine)

Primary function: Link two groups either by covalent binding or through reactive groups to improve the drug or vaccine (e.g., improve immunogenicity or targeting) (**Table 9.13-1**).

Table 9.9-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Ultrafiltration	Buffer composition and concentration Transmembrane pressure	Product concentration
Diafiltration	Buffer composition and concentration Transmembrane pressure Diafiltration volumes	Product concentration Product yield Purity pH Osmolality

Table 9.10-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Filling	Flow rate Load Factor (Filtration)	Product concentration (including uniformity across fill) Product yield Purity

Table 9.12-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Examples: Trypsin, Benzonase	Load factor Temperature Reaction time	Product yield Purity

Table 9.13-1 Example: Process Parameters and Performance Indicators

Sub-Unit Operation	Process Parameters	Performance Indicators
Conjugation	Concentration Temperature Conjugation time	Product homogeneity or polydispersity Conjugate size

Process Validation: A Lifecycle Approach

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