



Guide

Biopharmaceutical Process Development and Manufacturing



TECHNOLOGY FOR PRODUCTION, FINANCIAL, QUALITY AND COMPLIANCE SUCCESS



- Process Characterization / Design of Experiments (DOE)
- Site-to-Site Tech Transfer
- Scale-Up Tech Transfer
- Automation IT (Integration & IV&V)
- Verification, Commissioning & Qualification
- Process Validation
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Guide

Biopharmaceutical Process Development and Manufacturing

Disclaimer:

This Guide focuses on the development and the process approaches and practices involved in providing cost effective, regulated manufacturing of biopharmaceutical products in a timely manner that meet their intended use. ISPE cannot ensure and does not warrant that a system managed in accordance with this Guide will be acceptable to regulatory authorities. Further, this Guide does not replace the need for hiring professional engineers or technicians.

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Preface

The development and scaling up of processes is a cornerstone of success for the biopharmaceutical industry in making safe and effective medicinal products. These processes are required to meet cGMP regulations wherever products are marketed, while remaining in compliance with all other governing codes, laws, guidelines, and regulations.

This Guide focuses on the development and the process approaches and practices involved in providing cost effective, regulated manufacturing of biopharmaceutical products in a timely manner that meet their intended use. It is intended to be used by industry for the design, development and scaling up to regular production, all leading to processes meeting the requirements from FDA, EMA, MHLW, WHO, PMDA or other health authorities.

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1 Introduction

1.1 Background

The development and scaling up of processes is a cornerstone of success for the biopharmaceutical industry in making safe and effective medicinal products. These processes are required to meet cGMP regulations wherever products are marketed, while remaining in compliance with all other governing codes, laws, guidelines, and regulations.

There is a high focus on process understanding both from regulators and from the industry. A better process understanding is required under the currently much discussed concepts, e.g., quality risk management or quality by design, as outlined in respective ICH documents.

1.2 Purpose

This Guide focuses on the development and the process approaches and practices involved in providing cost effective, regulated manufacturing of biopharmaceutical products in a timely manner that meet their intended use. It is intended to be used by industry for the design, development and scaling up to regular production, all leading to processes meeting the requirements from the following:

- World Health Organization (WHO)
- European Medicines Agency (EMA)
- Food and Drug Administration (FDA) (US)
- Japan Ministry of Health, Labour, and Welfare (MHLW) (Japan)
- Pharmaceuticals Medical Devices Agency (PMDA) (Japan)
- Other health authorities

The Guide is intended to be in alignment with ICH guidance including:

- ICH Q3 [1]
- ICH Q4 [2]
- ICH Q5 [3]
- ICH Q6 [4]
- ICH Q7 [5]
- ICH Q8 [6]
- ICH Q9 [7]
- Applicable parts of ICH Q10 [8] and ICH Q11 [9]
- Associated international standards, regulations, and guidance documents

It is intended that all guidance offered is compatible with that provided by ASTM E2500 [10], and that if there is any perceived incompatibility, it is not deliberate.

This Guide is neither a standard nor a regulation. It is not intended to replace governing laws, codes, standards, or regulations that apply to process of this type. These are mentioned only for completeness and where their impact affects processes, equipment, or utility design affected by cGMPs.

The use of this Guide for new and existing processes, equipment, or utilities and associated automation is at the discretion of the owner or the operator.

1.3 Scope

This Guide intends to present a universal roadmap to process development and manufacturing of biopharmaceuticals that provides practical, scientifically sound guidance that helps users incorporate good practice and insight in order to develop robust processes that produce safe and more cost effective biopharmaceuticals. This guidance should be useful to persons involved in these activities, including process development scientists and engineers; manufacturing, quality, and regulatory personnel; and industry suppliers.

An important secondary goal is to incorporate current regulatory guidance, where possible, to further increase the efficiency of development activities and enhance compliance in the development and manufacturing of biopharmaceuticals. The integration of these two objectives provides a synergy that enhances the capabilities and effectiveness of industry professionals.

This Guide focuses on the scientific and process engineering principles associated with the design, development, optimization, and implementation of processes that are used in the manufacture of biopharmaceutical drug substance, also known as drug substance. It is neither a standard nor a GMP regulation. It is not intended to replace governing standards, laws, codes, or regulations that apply to biopharmaceutical processes of this type. The application of the concepts presented in this Guide for the development and design of manufacturing processes is at the discretion of the manufacturer.

This Guide applies to the development, design, and manufacture of biopharmaceutical drug substances. Specifically, it applies to the class of products that include protein therapeutics, monoclonal antibodies, and/or those cells or organisms that have been generated or modified by recombinant DNA/RNA, or other technologies to produce drug substance. It also may be applied to cell culture based vaccine products.

The concepts presented in this Guide represent a continuum of process design principles for products that are manufactured for clinical trial use as well as commercial scale production. This Guide applies to biopharmaceutical products that are regulated by the Center for Biologics Evaluation and Research (CBER) and the Center for Drug Evaluation and Research (CDER) at the US FDA.

This Guide fundamentally addresses current US FDA GMPs. Also it includes commentary from other countries and regions regarding GMP compliance. US National Institutes of Health (NIH) and World Health Organization (WHO) requirements are referenced where applicable.

The audience for this Guide is professionals involved in the design, development, implementation, operation, quality control, and quality assurance of biopharmaceutical processes. This includes regulatory personnel with a need to understand fundamental biopharmaceutical processes. It is the manufacturer's responsibility to justify decisions and approaches to regulatory authorities.

This Guide is not intended to be a comprehensive document that can be applied to all biopharmaceutical processes. It also does not guarantee that regulatory compliance will be achieved, but is intended to be used as a road map to assist in the decision making process related to the design, development, and manufacture of biopharmaceutical drug substances.

This Guide also does not address facility design issues that are covered in detail under the content of the ISPE Baseline® Guide: Biopharmaceutical Manufacturing Facilities (Second Edition) [11].

This Guide does not include details on:

- Transgenics
- Bio-safety level 3 and 4
- Combination products
- Human plasma fractionation

However, there are many similarities with the processes covered, why many aspects may be used also in these areas.

1.4 Key Concepts

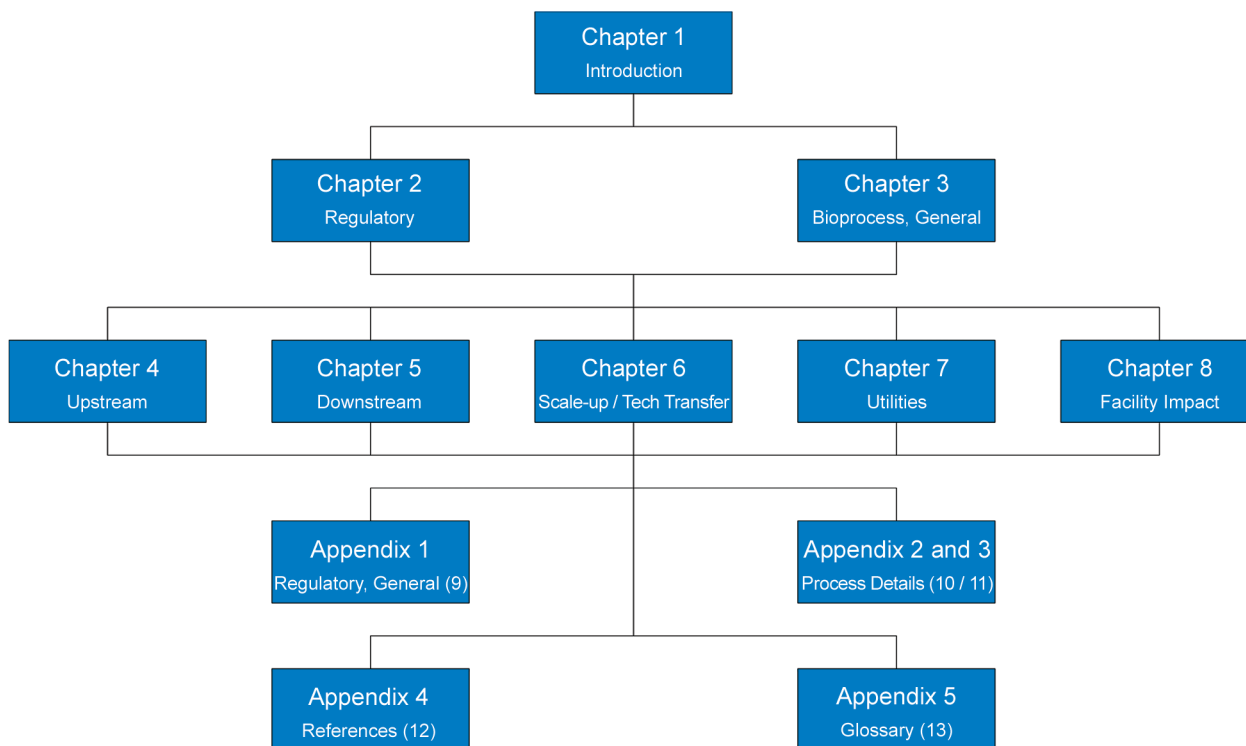
A key concept of biopharmaceuticals is that products are uniquely influenced by the manufacturing process (e.g., cell line, purification methodology) so fundamental understanding of the manufacturing process applied to process control is required to assure patient safety.

Biopharmaceuticals are typically large molecules and may be injected for therapeutic benefit. They are typically heat labile, cannot be terminally sterilized, and should be manufactured aseptically; therefore, manufacture of biopharmaceutical products poses a relatively high risk to patients and close attention to manufacturing control is appropriate. The following are key concepts and definitions that are central to this ISPE Guide:

- Regulatory aspects on the biotech processes
- Road map how to do process design and optimization
- Focus on processes and product, also from the scientific and operational aspects
- Principles of the various processes and unit operations
- Identification of critical process characteristics
- Discussion on single use versus conventional manufacturing

1.5 Structure

Figure 1.1: Guide Structure



2 Regulatory Considerations for Biopharmaceutical Process Development and Manufacturing

2.1 Introduction

Biopharmaceutical process development and manufacturing involves continual changes and improvements throughout the product life cycle.

Decisions regarding manufacturing processes which will lead to the best outcomes in terms of product quality and yield, are usually made early in process development and are based on the current scientific knowledge of a product.

The use of a “platform” manufacturing process that is based on knowledge gained over time from processes of previous products is widespread within the pharmaceutical industry. As development of biopharmaceutical products advances; however, the need for a non-platform process may arise. The formulation of a product also may change over time.

Biological medicinal products may consist of a number of product variants and process related impurities. It can be difficult to predict safety and efficacy profiles for these product variants and process related impurities, so assuring the quality of biological medicinal products can be challenging.

Safety issues are more often related to the mechanism of action or to the immunogenicity of a biological product, rather than to product quality, as most biopharmaceutical products are well characterized.

The Regulatory Affairs (RA) function can provide assessments and paths forward with regard to Chemistry, Manufacturing, and Controls (CMC) changes made throughout a product life cycle, including the impact of these changes in terms of:

- Expectations from guidance documents
- Requirements from regulations
- Changes to existing regulatory submissions
- The need for new submissions in support of the changes

RAs can reduce the level of regulatory risk associated with biopharmaceutical manufacturing and process development by:

- Championing a science- and risk-based approach that is well executed and well communicated
- Increasing the efficiency of specific product teams and the overall development process
- Ensuring submission of high quality applications that have the potential for shorter review times
- Improving positive relationships with regulatory agencies

GMP regulations apply for the markets in which the products are intended to be sold. For any safety, environmental and engineering legislation and guidelines referenced, the laws and guidelines in the country in which the development or manufacturing operation is taking place will apply.

2.2 Regulatory Organizations

The section highlights some of the principle GMP guidelines and regulatory authorities in operation around the world, including:

- World Health Organization (WHO)
- International Conference on Harmonisation (ICH)
- Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (PIC/S)

It also describes some of the specific GMP guidelines and authorities applicable to specific countries, as well as describing the harmonisation activities within specific regional areas.

2.2.1 *World Health Organization*

The WHO has established detailed guidelines for Good Manufacturing Practice (GMP). These GMPs are used by pharmaceutical regulators and the pharmaceutical industry in more than one hundred countries worldwide, primarily in the developing world. Several member states of the WHO have formulated requirements for GMPs, some of which are based on the WHO GMPs. Other countries have harmonized their requirements between countries, e.g., within the European Union (EU) or the Association of South-East Asian Nations (ASEAN); some countries have harmonized through (PIC/S).

Compliance with WHO standards alone does not fulfil the requirements to sell products in markets where regional regulations exist (such as in the US, EU, or Japan). Compliance with GMP regulations for the intended market is needed in order to sell products in these locations.

Compliance with regional GMPs (e.g., US, EU, and/or Japanese GMPs) does not imply compliance with WHO GMP regulations in their entirety. The WHO regulations should be reviewed for specific requirements in other global markets. The WHO GMP Guidelines can be accessed via the WHO website [12].

2.2.2 *The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has established Quality Guidelines. Guidelines, once adopted, are published in the three regions, i.e., Europe, Japan, and United States, and can be accessed via the ICH website [13].

2.2.3 *PIC/S*

The purpose of PIC/S is to facilitate the networking between participating authorities and the maintenance of mutual confidence, the exchange of information and experience in the field of GMP and related areas, and the mutual training of GMP inspectors. For further information, see the PIC/S website [14].

PIC/S has published a PIC/S GMP Guide (PE 009-9) [15] which has been adopted by some countries (such as Singapore and Australia) as their national GMP regulations.

2.2.4 *FDA*

The Public Health Service (PHS) Act and the Food, Drug, and Cosmetic (FD&C) Act [16] provide the statutory authority for the regulation of biological products in the United States (US). The Patient Protection and Affordable Care Act of 2010, as amended by the Healthcare Care and Education Reconciliation Act of 2010 established an approval pathway for follow-on biologics (biosimilars) in the US.

Food, Drug and Cosmetic Act

The FD&C Act [16] has its origins in the Food and Drugs Act originally approved in 1906 [17]. Significant revisions were made in 1938 and 1962. This broad-ranging law provides regulatory oversight for food, drugs, and cosmetics. Relevant regulations propagated from the FD&C Act for therapeutic protein products include those for current Good Manufacturing Practice (cGMP) in 21 CFR Part 210 [18] and 21 CFR Part 211 [19], as well as 21 CFR Part 312 [20] for submission of an Investigational New Drug Application (IND).

The FD&C Act specifically states that biological products are also drugs and must meet the requirements of the FD&C Act, when not in contradiction to the PHS Act [21].

2.2.4.1 Public Health Service (PHS) Act

The origins of the PHS Act go back to the Biologics Control Act of 1902 [22]. The PHS Act, as currently applied, was approved in 1944 and covers biological products and trivalent organic arsenicals. The term “biological product” refers to any virus, therapeutic serum, toxin, antitoxin, or analogous product applicable to the prevention, treatment, or cure of diseases or injuries of man. Therapeutic proteins and monoclonal antibodies are considered to be “analogous products.”

Section 351 of the PHS Act [21] provides for the regulation of biological products through licensure of establishments and products, labeling, right of inspection, and revocation. Section 352 of the PHS Act [21] provides for the manufacture of biological products by the US Public Health Service when otherwise unavailable.¹

Regulations propagated to implement the PHS Act [21] are found in 600 through 680, with 21 CFR Parts 600 – 606 being the most relevant for therapeutic protein products and monoclonal antibodies. Therapeutic proteins are exempt from some requirements of the 600’s as listed in 21 CFR Part 601.2(c)(1) [23].

Drugs under the jurisdiction of the PHS Act [21] also are also under the jurisdiction of the FD&C Act [16]. The PHS Act takes precedence over the FD&C Act.

2.2.4.2 Pharmacopoeias and Pharmacopoeial Harmonization

The United States Pharmacopeial Convention (USP) [24] provides standards for:

- Medicines
- Dosage forms
- Drug substances
- Excipients
- Medical devices
- Dietary supplements

It provides detailed information on several basic quality control testing procedures that can be implemented by pharmaceutical organizations with limited additional product-specific qualification. Some USP General Chapters are directly applicable to biopharmaceuticals; therefore, the FDA expects these to be followed (with a few exceptions). Appropriate alternatives also may be acceptable.

¹ Section 352 of the PHS Act [21] Allows the PHS to prepare biologics for personal use or for public use if not available otherwise.

A number of methods published by the USP [24], the European Pharmacopoeia (EP) [25], and the Japanese Pharmacopoeia (JP) [26] were harmonized under the auspices of the ICH via the Pharmacopoeial Discussion Group (PDG). These harmonized methods are usually acceptable to the regulatory agencies of the US, the EU, and Japan. For a specific list of harmonized methods and their ICH stage of implementation see ICH Q4 [2].

2.2.4.3 *Product Jurisdiction*

The FDA uses a patchwork series of departments and processes for the approval of products derived from biological sources. Depending on their size, function, and origin, biotechnology-derived products may be approved via:

- A New Drug Application (NDA) with CMC review occurring in the FDA Office of New Drug Quality Assessment (ONDQA) within CDER
- Within the Office of Biotechnology Products (OBP), also within CDER

Products also may be regulated via:

- A Biologic License Application (BLA) in OBP/CDER
- The Office of Blood Research and Review (OBRR) in the Center for Biologics Evaluation and Research (CBER)

A typical monoclonal antibody product is approved via a BLA and reviewed by OBP/CDER.

Questions on jurisdiction can be directed to the Regulatory Project Manager assigned to an application. Where an application has not yet been submitted, available information can be reviewed on the FDA website, the office most likely to review a product, or the US FDA Office of Combination Products, can be contacted.

The US Office of the Ombudsman is available to help resolve jurisdiction issues.

2.2.5 *European Union*

The EMA was set up by European Community (EC) Regulation No. 2309/93 as the European Agency for the Evaluation of Medicinal Products, and renamed by EC Regulation No. 726/2004 to the European Medicines Agency. For further information see the EMA website [27].

The biological products described in this Guide would be reviewed for marketing authorization via the centralized procedure wherein the application is sent to the EMA. However, products in development are generally approved for use in clinical trials by submitting a Clinical Trial Application (CTA) and associated Investigational Medicinal Product Dossier (IMPD) to individual National competent authorities. An application is usually required for each country in which a sponsor wants to initiate a clinical trial. However, the Voluntary Harmonisation Procedure (VHP) for clinical products intended for a minimum of three European countries, allows sponsors to submit a CTA to a central office and receive only one set of comments. Although the VHP may not save time in regard to an approval, it may save effort in submitting the same application to several countries and replying to several sets of questions.

The ICH Common Technical Document (CTD) can be submitted to both the EMA and to National Regulatory Authorities. Points of note about an IMPD for review in European countries include:

- Analytical method validation is expected earlier than in the US
- Section 3.2.P.2 (Pharmaceutical Development) may need to be more detailed than in the US
- There can be different expectations for release and stability specification limits, though these can be reasonably easily aligned to meet the expectations of both the EU and US.

- A more detailed Facility Summary is required, but similar to specifications, a section can be designed to meet the expectations of both the EU and US.
- QP and GMP Certification are required. In the absence of a GMP certification process in the US similar to that in the EU, this can be a challenge.
- Bovine Spongiform Encephalopathy (BSE)/Transmissible Spongiform Encephalopathy (TSE) Certificates are required.
- The requirements for extending shelf-life of products vary from country to country in the EU and differs from that in the US. In the EU, some countries require a substantial amendment to extend shelf-life. Some countries require an expiry date on the Drug Product label. This lack of harmonization may be challenging when a sponsor aims to start clinical trials as quickly as possible and can be challenging logistically when submissions and re-labeling are required. In the US, expiry dates are not required on Drug Product labels and a wait period for approval of an amendment to extend expiry is not established.

2.2.5.1 *Genetically Modified Organisms and Genetically Modified Microorganisms*

The EU is generally more focused on this issue of Genetically Modified Organisms and Genetically Modified Microorganisms (GMOs and GMMs) than the US. The European legislation on the contained use of genetically modified microorganisms is 2009/41/EC [28]. This legislation is implemented nationally by the Environmental Regulatory Organization within each country. The “Contained Use” system is applicable to the biopharmaceutical industry where the operations take place in closed equipment, inside buildings which provide secondary containment. “Uncontained Use” applies to the use of GMOs in Agriculture.

2.2.6 **Asia, Australia, and Pacific Region Regulatory Authorities**

Countries in Asia generally implement GMPs at a national level. Through initiatives such as the Asia Pacific Economic Cooperation (APEC) some countries have implemented the PIC/S GMP Guidelines [15] as their national standards (such as Australia and Singapore), others utilize the WHO GMP Guidelines [12] (such as Vietnam), and others implement their own national Guidelines, including:

- **India:** The Drugs and Cosmetics Act (1940) and the Drugs and Cosmetic Rules (1945) – Schedule M (GMP Practices and Requirements of Premises, Plant and Equipment for Pharmaceutical Products) For further information, see the Central Drugs Standard Control Organization website [29].
- **China:** Good Manufacturing Practice for Pharmaceutical Products. For further information, see the State Food and Drug Administration (SFDA) [30].
- **Japan:** Standards for Manufacturing Control and Quality Control of Drugs and Qasi-Drugs (GMP Ministerial Ordinance no. 179). For further information, see the Ministry of Health, Labour and Welfare website [31] and the Pharmaceutical and Medical Devices Agency (PMDA) website [32].

There are also several industry organizations which offer guidance on GMP regulations in different regions, e.g., the Japan Pharmaceutical Manufacturers Association (JPMA) in guidance for the Japanese market regulations. For further information, see the JPMA website [33].

2.2.7 **Americas (Non-US)**

Through the Pan American Network for Drug Regulatory Harmonization (PANDRH) Working Group on Good Manufacturing Practice; within this organization a Pan American Health Organization (PAHO) Guideline for Good Manufacturing Practices Inspections (based upon WHO Report No. 32) has been published, and other documents are issued for public opinion. For further information, see the PAHO website [34].

2.2.8 Middle East

The Gulf Central Committee for Drug Registrations (GCC-DR) has responsibility for central drug registration and inspection of pharmaceutical facilities for its member states which include Saudi Arabia, Kuwait, Oman, United Arab Emirates, Bahrain, Qatar and Yemen. The GCC has published General GCC Guidelines for Good Manufacturing Practice for various types of Medicinal Products. For further information, see the GCC-DR website [35].

2.2.8.1 Commonwealth of Independent States

The Commonwealth of Independent States (CIS) countries include Armenia, Azerbaijan, Belarus, Kazakhstan, Kyrgyzstan, Moldova, Russia, Tajikistan, Turkmenistan, and Uzbekistan. (Some countries are already covered under Europe.)

2.2.9 Africa

Most African countries (such as Kenya) observe the WHO GMP Guidelines, while South Africa has been a member of PIC/S since 2007.

The Southern African Development Community (SADC) is developing a free trade area, which is expected to ultimately include GMP Harmonization activities. For further information see the SADC website [36].

2.3 Specific GMP Regulatory Requirements for Non-US Markets and Non-US Manufacturing Locations

2.3.1 Room Classifications

Room classifications will impact upon biologic processes and products where open processing is employed in the manufacturing process.

2.3.1.1 Europe

European GMP Regulations currently use a room grade classification which denotes the room grade in both “at rest” and “in operation” conditions. The relationship between the ISO room grades is outlined in the Table 2.1.

Table 2.1: The Relationship Between the ISO Room Grades

ISO 14644-1 [37]			EU GMP Annex 1 [38]				
ISO Category	Maximum Concentration (particles/m³ of air)		EU Grade	Maximum Concentration (particles/m³ of air)			
				In Operation		At Rest	
	≥ 0.5 µm	≥ 5.0 µm		≥ 0.5 µm	≥ 5.0 µm	≥ 0.5 µm	≥ 5.0 µm
ISO 5	3520	29	A	3520	20	3520	20
			B			3520	29
ISO 7	352,000	2,930		352,000	2900		
			C			352,000	2,900
ISO 8	3,520,000	29,300		3,520,000	29,000		
			D			3,520,000	29,000
				Not Defined	Not Defined		

It should be noted that while the comparison is close in the table above, it is not exactly equal for the number of particles in the $\geq 5.0 \mu\text{m}$ range.

In addition, the European GMP regulations provide limits for microbiological monitoring of clean areas during operation, in accordance with Table 2.2.

Table 2.2: European GMP Recommended Limits for Microbial Contamination^(Note 1)

Grade	Air Sample cfu/m ³	Settle Plates (diameter 90 mm) cfu/4 hrs ^(Note 2)	Contact Plates (diameter 55 mm cfu/plate)	Glove Print 5 fingers cfu/glove
A	< 1	< 1	< 1	< 1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-
Notes: 1. These are average values 2. Individual settle plates maybe exposed for less than 4 hours				

2.3.1.2 India

The Indian regulation also defines the room classification in both “At rest” and “In Operations,” but the limits are lower than that of EU, most notable is the 5 micron particle limit being “0” in Grade A both in operation and at rest condition.

Table 2.3: Indian GMP Requirements for Airborne Particulate Classification for Manufacture of Sterile Products

Grade	Maximum Number of Permitted Particles per cubic meter equal to or above			
	At Rest		In Operation	
	0.5 μm	5 μm	0.5 μm	5 μm
A	3500	0	3500	0
B	3500	0	3,50,000	2,000
C	3,50,000	2,000	35,00,000	20,000
D	35,00,000	20,000	Not defined	Not defined

2.3.1.3 Japan

Japanese GMP regulations do not utilize a room grade classification, such as European GMP Regulations. It is only shown in current Japanese Pharmacopeia (JP) [26].

The room classification system in use in Japan is outlined in Table 2.4.

Table 2.4: Japanese GMP Air Cleanliness Requirements for Processing of Sterile Pharmaceutical Products

Air Cleanliness	Maximum Number of Airborne Particulates per m ³	
Grade ^(Note 1)	At Rest	In Operation
	≥ 0.5 µm	≥ 0.5 µm
A (Laminar-airflow zone)	3,530	3,530
B (Non laminar-airflow zone)	3,530	353,000
C	353,000	3,530,000
D	3,530,000	--- ^(Note 2)
Notes: 1. The maximum permitted number of particles in the “in operation” condition corresponds to the standards described under USP <1116> [24] as follows: Grade A: Class 100 (M3.5); Grade B: Class 10,000 (M5.5); Grade C: Class 100,000 (M6.5); Grade D: no corresponding standard. 2. The limit for this area will depend on the nature of the operation carried out there. <i>(Citation from Table 1 from the Japanese Pharmacopeia, Fifteenth Edition, General Information Chapter, Section 13: Microbiological Evaluation of Process Areas for Sterile Pharmaceutical Products.)</i>		

Additionally, the JP XV [26] provides limits for microbiological monitoring of clean areas during operation, in accordance with Table 2.5.

Table 2.5: Japanese GMP Recommended Limits for Environmental Microorganisms^(Note 1)

Grade	Airborne Microorganisms ^(Note 2) (CFU/m ³)	Minimum Air Sample (m ³)	CFU on a Surface	
			Instrument/Facilities (CFU/24-30 cm ²) ^(Note 3)	Gloves (CFU/24-30 cm ²) ^(Note 3)
A	< 1	0.5	< 1	< 1
B	10	0.5	5	5
C	100	0.5	25	---
D	200	0.5	50	---
Notes: 1. Maximum acceptable average numbers of microorganisms under each condition. 2. These values are by using a slit sampler or equivalent. 3. Viable microbe cell number per contact plate (5.4 to 6.2 cm in diameter). When swabbing is used in sampling, the number of microorganisms is calculated per 25 cm ² . For gloves, usually, put their all finger on the plate.* <i>(Citation from Table 4 from the Japanese Pharmacopeia, Fifteenth Edition, General Information Chapter, Section 13: Microbiological Evaluation of Process Areas for Sterile Pharmaceutical Products.)</i>				

* I.e., for gloves, an impression of all fingers should be taken on the plate.

2.3.2 Viral Reduction/Clearance

Generally Viral Reduction in biopharmaceutical processes will follow the requirements of ICH Q5A(R1) “Note for Guidance on Quality of Biotechnological Products: Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin,” Chapter 6: Evaluation and Characterisation of Viral Clearance Procedures [3].

As a supplemental requirement, the European GMP/GDP working group has published a draft version of EU GMP Annex 2 [40]. This states:

“For products that are inactivated by the addition of a reagent, the process should ensure the complete inactivation of live organism. In addition to the thorough mixing of culture and inactivant, consideration should be given to contact of all product-contact surfaces exposed to live culture and the transfer to a second vessel”

This suggests that potentially a second vessel may be required as part of any acid addition viral reduction step.

Indian GMP regulations do not currently have any specific requirement for viral reduction in biotechnology derived products.

2.3.3 Residual DNA

The limit of residual DNA content in the final product permitted by the EU and the WHO is up to 10 ng per dose depending upon the source of the residual DNA and the product’s route of administration. The industry standard in the US is specified in FDA Guidelines and is 100 pg per dose.

In China, residual host DNA quantities generally follow the regulatory Guidelines of EU, FDA, and WHO, but for vaccines in China, the acceptable residual amount of DNA is more strict and is set on a case by case basis, for example:

Table 2.6: Acceptable Residual Amount of DNA in China

Domestic	Product Name	Cell	Standard
	Rabies Vaccine	Vero	100 pg/dose
	Hemorrhagic Fever with renal syndrome vaccine	Vero	100 pg/dose
	Japanese encephalitis vaccine	Vero	10 pg/dose
	Hepatitis A inactivated vaccine	Vero	10 pg/dose
	Hepatitis B vaccine	CHO	10 pg/dose
Imported	Infantile Paralysis Vaccine (IPV)	Vero	10 pg/dose
	Rabies Vaccine	Vero	10 ng/dose

The residual DNA limits for vaccines in China are listed in the China Pharmacopoeia 2010 edition [39].

The Japanese Ministry of Health, Labor and Welfare (MHLW) have adopted ICH Q6B Guideline “Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products” and legislated one of the Japanese Guidelines on 1 May 2001. Japanese regulation has no limit of residual DNA content because ICH Q6B [4] did not show the limit in final product. Japanese companies may refer to EU/WHO acceptance criteria (10 ng/dose) and establish own limit.

2.3.4 Buffer Preparation for Final Bulk Biologic Drug Substance Manufacturing Steps

The EU initially published a draft version of Annex 2, [40] which infers by reference to Annex I, that the final buffer used in the manufacture of a bulk biologic drug substance will should be dispensed and prepared in a room with a grade C background. The latest draft of the new annex (March 2010) does not explicitly reference this subject anymore, nonetheless this matter should be considered in the risk assessment for the product on the basis that the final buffer is processed within any downstream fill finish facility, which has this requirement for any buffers used.

2.3.5 Filter Integrity Testing

There is an EU GMP Requirement to integrity test filters after they have been sterilized before use, due to concerns over possible damage during autoclaving or SIP of the filter, and not detecting the damage afterward due to re-blocking during filtration. It is noted that this is an Annex 1 (sterile processing requirement) [38] and occurs in the section related to “filtration of medicinal products that cannot be sterilized into their final container.” It is also noted that some EU regulators are now requesting biopharmaceutical companies to consider pre-use *in-situ* integrity testing of filters around bioreactors and fermenters which provide the sterile boundary prior to introduction of the media and organisms for axenic growth. While not explicitly in the latest volume of Annex 2 [40], it is recommended that this potential requirement is discussed with the appropriate EU GMP regulator.

2.3.6 Purified Water and Water for Injection

The Indian GMP requires that Water for Injection (WFI) is generated by distillation process only, and needs to be maintained above 70°C, and that distribution lines for Purified Water (PW) and WFI are required to be of 316 SS.

2.3.7 Drug Development and Clinical Trials

In the US, an Investigational New Drug (IND) application is prepared and submitted to the FDA, while in the EU a CTA is prepared and submitted to the EMA and National Regulatory Authorities. The science is the same, and the applications CTA and IND documentation requirements are mostly the same although there are some minor differences. The CTD format is the same worldwide. Some points to note about the EU application:

- IMPD CMC differences include:
- Analytical method validation early (in the US this happens during Phase III)
- Expects Section 3.2.P.2 (Pharmaceutical Development) (not addressed in the US CMC, but generally considered).
- Different release and stability specification limits
- Facility Summary
- Qualified Person (QP) and GMP Certification
- TSE/BSE Certificates

In Asia it is noted that new drug substances discovered in India need to go through all phases of clinical trials in India. For new drug substances discovered outside India, Phase I clinical data generated outside India is required for permission to initiate Phase I or Phase II clinical trials. Phase III clinical trials are required to be conducted in India before permission to market the drug in India is granted.

In Japan, the MHLW adopted ICH GCP Guideline E6(R1) [41] and enacted it as a Japanese GCP Guideline in 1996 and amended it on 2007; therefore, the Japanese GCP Guideline [42] has been harmonized with international regulation.

2.4 Relationship to ICH Guidance Documents

2.4.1 FDA and ICH Guidance Documents

The US FDA may publish guidance that further clarifies the intent of the PHS Act [21] and the FD&C Act [16] beyond that found in the associated propagated regulations. Guidance reflects the current thinking of the FDA on a given topic, but it is not binding. Other approaches than those discussed in the FDA guidance can be used, if justified. Current guidance is normally located on the US FDA website [43].

ICH also publishes guidance specific to the development, manufacture, and testing of pharmaceuticals. Representatives from industry and regulatory agencies from the US, the EU, and Japan are members of this joint initiative. As stated on the ICH website [13]: “The ICH Guidelines are not intended to be comprehensive guidance covering all aspects of product development and registration. They are intended to be used in combination with any regional requirements.”

Some ICH documents, particularly the ICH Q5 series [3], are specific to biotechnology-derived products and some apply to both large and small molecules.

2.4.2 Europe (Eudralex) and ICH Guidance Documents

Also it should be noted that as each country European regulator may have specific requirements above those contained in the European GMPs that a preconstruction regulatory briefing meeting with the relevant EU regulator is generally recommended to establish any specific requirements from that regulator.

It is also noted that ICH Guidelines, once adopted by Europe, the US, and Japan are enacted within the European (and US and Japanese) GMP standards although sometimes this is not on a totally identical basis. For example, in the case of ICH Q7a [5], the standard was initially transferred to the EU regulation by amending the EU GMP Guide [44] (Annex 18). The content of Annex 18 is today called Part II of the EU GMP Guide [44]. However, a revised Part II text for the EU GMP Guide was brought into force on 31 July 2010 which identified “An amendment is made to Part II of the GMP Guide to incorporate principles of Quality Risk Management in line with the ICH Q9 [7] Guideline on Quality Risk Management. Amendments correspond to similar changes made to Part I Chapter 1 of the Guide and published in February 2008. A new section on Quality Risk Management is introduced as section 2.19. The remaining sections of chapter 2 are renumbered. A minor change is made to section 2.21. No other changes have been made.”

It also should be noted that not all countries in Europe are members of the EMA.

2.5 Regulatory Considerations Across the Product Life Cycle

At the start of a clinical product program, a product development strategy should be created. Program planning should include a cross-functional approach with the goal of product launch. Regulatory strategies should be based upon the overall product development strategy, and should be designed to maximize the program planning process and reduce risks to the program. Product development strategies are likely to have several iterations; therefore, appropriate strategies for each stage of development should be considered.

Life cycle management should begin with the development of a Target Product Profile (TPP) and then a Quality Target Product Profile (QTPP) to help maximize effectiveness.

In March, 2007 the FDA issued draft guidance for industry entitled “Target Product Profile – A Strategic Development Process Tool” [45]. The TPP usually begins with sponsors providing the proposed labeling concepts that are the goal of the drug development program. From a CMC perspective, the TPP is useful in designing the product to meet its intended use requirements. For example, if a sponsor is developing a product to treat rheumatoid arthritis, the TPP could describe clinical and nonclinical studies with a subcutaneous injection route. The CMC team may be directed to develop a container closure format to be self-administered subcutaneously. Based upon this knowledge, the CMC team can then define the path toward reaching the proposed marketing configuration for the pivotal study.

Other considerations linking back to the TPP include the proposed patient population and an overall global development plan. Countries (e.g., Japan) may require early phase 1 data from the relevant ethnic population, may have specific regional requirements or may have different interpretations of ICH guidance.

The QTPP is defined in ICH Q8(R2) [6] as:

“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.”

The QTPP is derived from Quality by Design (QbD) initiatives and is developed after the TPP. Whereas the TPP is intended to help to focus the broader development team toward marketing goals, the QTPP is specific to product quality attributes. The QTPP is intended to help to focus the CMC team on the final product quality goals, as well as the final product delivery goals (i.e., product concentration and packaging; vial, pre-filled syringe, etc.)

2.5.1 Acceptance Criteria or Specifications

According to ICH Q6B [4], a specification is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria.

Specifications should be set at the time of the original IND submission to ensure safety, purity, and potency. Specific requirements, such as those for safety (e.g., sterility, endotoxin, host cell DNA) are moderately prescribed. The setting of acceptance criteria for other attributes can be challenging, based on the limited information generally available for the process at this stage of development. These acceptance criteria are usually comparatively broad. In early development, it may be acceptable to have “report result” as an acceptance criterion, depending on the assay. As clinical development progresses and manufacturing experience is gained, quantitative acceptance criteria should be established. Quantitative acceptance criteria are expected wherever possible.

Acceptance criteria such as “conforms to reference standard” may be acceptable, provided that the quality attribute of the reference standard for that method is well defined. For example, for native capillary isoelectric focusing and gel electrophoresis (reducing and non-reducing) the number and pI range or size of bands or peaks should be quantified in addition to stating “conforms to reference standard.”

For impurities, a justification that the product is safe for its intended use is normally required. This justification should consider the anticipated exposure of volunteers and the patient population, as defined within the TPP. Acceptance criteria may not reflect process capability, due to limited experience early in development; however, acceptance criteria may be based on prior product knowledge.

As knowledge and experience increases, analytical methods may need to be modified. Acceptance criteria set for previous trials should be reviewed and adjusted. Removal of a specification or widening of acceptance criteria should be submitted to the FDA and the cover letter should clearly state the reason for the submission. The FDA is not required to provide a response to IND amendments; however, the expectation for review and concurrence before implementing new acceptance criteria may not be realistic.

ICH Q6B [4] states that the assessment of purity may be highly method-dependent. Overall product purity and product-related substances/impurities should be evaluated by a combination of orthogonal analytical methods. A justification should be submitted within the BLA to support the selected analytical methods used for release and stability testing of both drug substance and drug product based on:

- Relevant development data
- The lots used in non-clinical and/or clinical studies
- Data from lots used for demonstration of manufacturing consistency
- Data from stability studies

Lack of appropriate testing of lots used in pivotal trials can present a significant BLA review challenge. Assays for preservative effectiveness, as required when developing a multi-use vial, should be validated and the information included in the BLA. In addition, methods for detection of impurities (host cell DNA, host cell proteins, residual Protein A, etc.) are usually required for Phase I lot release of drug substance; however, it is possible to demonstrate acceptable clearance of these impurities such that they no longer need to be monitored by lot release testing after approval.

2.5.2 Process Development

These early stages of process development are focused on safety rather than on GMP requirements.

In 2008, the FDA released the guidance document entitled “cGMP for Phase 1 Investigational Drugs” [46]. This guidance document was published at the same time as an update to 21 CFR Part 211 [19] which stipulated that some elements of cGMP are not required for exploratory IND or Phase I IND products. These updates essentially harmonized rules and guidance with actual industry practice during Phase I. For example, based upon this guidance, sponsors manufacturing Phase I investigational products are not required to have specific processes validated, if those processes are expected to be validated for a late stage product. This differs from the EU requirements for cGMP manufacturing at Phase I.

2.5.3 Comparability Assessments during Clinical Development

During the early stages of product development manufacturing changes can be frequent, particularly during the development of monoclonal antibodies and related products. Manufacturing processes and their control strategies are usually continually improved and optimized; these improvements and optimizations should be appropriately assessed and documented.

Numerous manufacturing changes can occur during Phase II. For example, where changes to the final formulation and/or container closure are needed, these should be implemented during Phase II. These types of changes should be avoided after the start of a pivotal study, due to concerns with establishment of comparability and adequate experience/exposure with the proposed final commercial product.

Quality attributes of a drug substance and relevant intermediates should be compared using suitable analytical methods, which may include an assessment of stability changes (i.e., rate and mode of degradation).

A comparability exercise may be needed to assess risks introduced by a manufacturing change, depending on the consequences of the change and the stage of development at which the change was introduced. These studies should provide assurance that the post-change product is suitable for clinical trials and will not raise any concerns regarding patient safety. Where accumulated experience and other relevant information and data are not sufficient to assess the risk introduced by the change or if a potential risk to the patient safety is anticipated, a comparability exercise based only on product quality attributes may not be sufficient.

After the start of pivotal trials, changes to manufacturing processes require robust assessment of comparability in order to ensure links to material used in previous clinical trials and, therefore, allow use of clinical data generated from earlier studies. Product used in the pivotal trials should be from only one process. Any change between lots when going into a pivotal trial can present an extremely high risk.

Aspects to consider for comparability exercises:

- Changes to raw materials may necessitate an assessment of comparability. Risk management principles can be useful and a discussion of the assessment should be included in regulatory submissions.
- Comparability of pre-clinical and clinical materials: should be linked. A risk assessment may be helpful in reviewing differences in the manufacture of pre-clinical and clinical materials.
- Comparability of process changes during clinical development:
 - The comparability of pre-change and post-change product should be shown
 - Associated clinical studies should be linked
 - Processes from which patients received material should be identifiable
 - A robust process naming convention can be helpful in identifying the specific process. A computer software versioning convention is recommended, e.g., Process 1.0. Small changes may lead to Process 1.1. More complex changes may lead to Process 1.5 and significant changes increase the version by a whole number, such as Process 1.0 to Process 2.0.
- Appropriate FDA and ICH guidance (e.g., ICH Q5E [3]) should be consulted when assessing the results from comparability exercises during clinical development. Reference should be made to available literature and prior product knowledge to support minor changes rather than moving on to non-clinical or clinical studies. It also should be recognized when product quality assessments are insufficient.
- Stability data, including assessments of the rate and mode of product degradation should be used to support comparability conclusions.
- As development progresses, more data is likely to be needed. Products in later stages of development may need additional studies (e.g., photostability studies or forced degradation studies) to support comparability.
- Cross-company information from conferences and white papers should be leveraged. Regulatory agencies can be requested to provide feedback on more complex issues.

2.5.4 Process Validation

The FDA's Guidance for Industry on "Process Validation: General Principles and Practices" [47] defines process validation as:

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

The FDA PV Guidance [47] emphasizes that process validation is a life cycle approach and should be applied by linking product and process development, qualification of the commercial manufacturing process, and maintenance of the process in a state of control during routine commercial production.

For further information on process validation, see ICH Q2(R1) Validation of Analytical Procedures: Text and Methodology [48], the EMA Guideline on Process Validation [49], the FDA PV Guidance [47], the ISPE Product Quality Lifecycle Implementation (PQLI) discussion papers on Process Validation [50], and the PDA TR-60: Process Validation: A Lifecycle Approach [51].

2.6 Differences Between the EU and FDA Approaches to Risk

A main difference between the FDA and EU regulations comes in the perception of risk, the use of a scientific basis, and how to manage the risk.

The FDA reviews specific data about consumer product complaints and uses a quantitative method of risk assessment. The risk assessment process involves five steps:

1. Stating the problem
2. Identifying the cause of the problem
3. Determining how much exposure has taken place
4. Assessing the response to the hazard
5. Determining the affected population's level of exposure

The EU regulatory system's risk assessment process employs a more qualitative method, based on the entire set of available data. The EU's process identifies hazards and population at risk, examines and prioritizes identified risks, creates a plan of prevention, takes action based on three previous steps, and finally assesses which part of the plan works and which does not.

Risk assessment methodologies for the purpose of a structured approach to reaching alignment on risk issues are described within ICH Q9 [7].

With regard to European legislation, the reader should be aware that European legislation is firstly enacted by the European Parliament and Council at a European level, this EU Legislation is known as EudraLex, and consists of 10 volumes, for which Volume 4 "Guidelines for Good Manufacturing Practices for medicinal products for human and veterinary use" [44] defines the EU GMP requirements.

This EudraLex legislation is then enacted (sometimes several years later) at a national/country level, national governments may supplement the European legislation either when they enact the legislation or subsequently through bulletins from the relevant national agency. For example, the United Kingdom GMP regulator is the Medicines and Healthcare Products Regulatory Agency (MHRA) which publishes the "Rules and Guidance for Pharmaceutical Manufacturers and Distributors" (also known as the Orange Guide) [52] which collates into one volume both European and UK guidance for GMP. National legislation and guidance (when in place) should always be referred to, as each national government has the right to enact additional legislation of their own specific to their own country.

3 Biopharmaceutical Processes, General

3.1 Introduction

This chapter aims to provide an overview of biopharmaceutical processes and unit operations, and outlines the stages of development and approval to bring a biopharmaceutical product to commercial realization. It addresses major unit operations along with activities that are required to support process development and commercialization, including:

- Process operations
- Unit operations
- Analytical support
- Stability activities
- Cleaning activities

Although manufacturing process and critical parameters can differ, platforms and unit operations for biopharmaceuticals may share some common process characteristics. Process considerations should consider organizational philosophies that can drive platform and product development.

Millions of compounds are usually screened for each commercially viable biopharmaceutical drug product. Biopharmaceutical drug candidates travel through a rigorous process for approval.

Following discovery and optimization, a IND/CTA is filed prior to commencing First in Human (FIH) studies for the biopharmaceutical drug candidates.

PRE-IND

The focus of process development during the pre-IND phase is to develop a manufacturing process that delivers a product that is safe and suitable for initiation of a Phase I clinical program. During this stage of development, preliminary material may be manufactured on a small scale to provide material for limited purposes such as:

- Preclinical studies
- Initial characterization
- Formulation selection
- Method development

Two aspects should be considered:

1. For nonclinical studies, traceability of manufacturing methods should be maintained so that data generated can be traced back to the actual manufacturing processes.
2. For formulation development, formulation screening studies (as a minimum) should be performed prior to final formulation selection. Inadequate exploration of an appropriate formulation early in development can delay the program later on. This exploration should be performed in the context of initial characterization of the molecule, and the proposed TPP and QTPP.

For cell culture processes, preliminary viral clearance studies should show clearance via orthogonal steps (different mechanisms for clearing viruses that are used in the same process).² EU guidance should be considered for platform approaches as duplicate runs on unit operations and at least two orthogonal steps are required (see the EMA Draft Guideline on Virus Safety Evaluation of Biotechnological Investigational Medicinal Products [53]).

Cell culture processes may use raw materials of a biological origin (e.g., sourced from animal components including bovine serum) to assist in cell growth. There is a concern with using animal derived material that it may be contaminated with BSE/TSE agents. Biological product manufacturers have been requested by regulatory agencies to determine the origin of these materials used in manufacturing, because there is no sensitive in vitro assay to detect the presence of these agents. Documentation should be provided in regulatory submissions regarding the safety of raw materials of biological origin.

However, the majority of new products do not have animal-derived components in any part of the production process to avoid risk of BSE and TSE contamination.

Once animal safety has been established, an IND/CTA can be filed at the start of Phase I, which is designed to establish safety in humans.

- Phase I data should be reviewed and approved by regulatory agencies.
- Phase II expands the clinical application to a larger patient enrollment with final data at end of Phase II reviewed by regulatory agencies.
- Phase III expands patient enrollment to assess human safety and efficacy of the biopharmaceutical drug candidate.
- Once approved, a biopharmaceutical drug candidate undergoes a registration process with regulatory agencies. This is normally followed by commercial introduction performed in parallel to ongoing surveillance.

Scale-up and technology transfer of the manufacturing process should support the drug candidate's clinical supply demand. Considerations for unit operation scale-up can have significant impacts on the commercial viability of a drug candidate and also can limit the ability to supply clinical trials. Scale up operations should consider QbD to streamline process development and optimization. See Chapter 6 of this Guide. The investment in process development and process and analytical qualification should be commensurate with the clinical stage and the probability of technical and regulatory success.

Unit operations may be used to obtain product attributes that will define the product. A suitable in-process control strategy should be developed in relation to the process parameters and Critical Process Parameters (CPPs) that control and monitor operations.

Analytical development activities should be consistent with the stages of process understanding, as the product progresses from Phase I through to Phase III and commercialization. Analytical assays that in a modular or generic approach for viral clearance.

Support the process are considered essential for:

- Development
- Scale-up
- Validation

² Some pharmaceutical organizations may support the initiation of clinical trials by leveraging prior product knowledge.

- Commercialization

A strategy should be developed to ensure the appropriate investment of resources in order to facilitate:

- Development
- QbD
- Troubleshooting
- Stability activities

The effort and time needed to develop analytical assays and to meet regulatory expectations, including the scope of testing, should be understood.

3.2 Stages of Biopharmaceutical Product Development

3.2.1 Development

Biopharmaceutical drug development involves several phases for drug approval. Drug development begins with research and development exploration. The length of time for discovery of a potential biopharmaceutical drug product can vary significantly and may take more than ten years. The development phase is intended to establish qualification parameters for a biopharmaceutical drug product, which leads into non-clinical studies.

3.2.1.1 Nonclinical Studies

Nonclinical (pre-clinical) studies involve animal and in-vitro studies performed to support future clinical studies. The studies should be performed in at least two species one may be a rodent and one a non-rodent (see the ICH S6(R1) Addendum to Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals [54]).

Nonclinical studies should evaluate “ADME”, i.e.:

- Absorption
- Distribution
- Metabolism
- Excretion

Biopharmaceutical drug candidates should be evaluated for safety, potential toxicity, and biological activity. In addition, chemical and physical characterization should be performed and qualitative and quantitative analytical methods should be developed. Nonclinical studies must show that a candidate is safe for early testing in humans, and is used to eliminate the majority of safety concerns before the product reaches human clinical trials. For further information on the regulatory requirements for nonclinical studies, see the US FDA, 21 CFR Part 58, Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies [55].

Nonclinical studies may continue until licensure and through the market life of the biopharmaceutical product.

Nonclinical studies should select only those candidates that are deemed to have a high probability of effectiveness. GLP practices should be maintained throughout early, preclinical development to assure that:

- Data are valid and correct
- A study can be repeated with identical outcomes
- The design, implementation, and documentation of the studies are maintained

The IND Application should be submitted. An IND is a request for authorization from the US FDA to administer an investigational drug or biological product to humans. For further information on requirements for submission of an IND, see 21 CFR Part 312, Investigational New Drug Application [20].

During nonclinical studies, data should be collected on safe and efficacious dosing regimens to be used in humans.

3.2.1.2 Phase I

Phase I clinical studies are usually performed in a small number of healthy volunteers. Participant numbers may range from 10 to 20 individuals. These studies are usually performed in both single and multi-dose regimes to evaluate safety and pharmacokinetics (the evaluation of the extent and duration of exposure to drug at various dose levels, routes and durations). For further information on requirements for human studies, see 45 CFR Part 46, Protection of Human Subjects [56].

CGMPs apply to the entire production process, from raw material to final product, for any approved drug, licensed biologic, or clinical trial material. For further information on requirements for making of the drug product, see 21 CFR Part 210 and 21 CFR Part 211, Current Good Manufacturing Practices (cGMP) in Manufacturing, Processing, Packing, or Holding of Drugs and Finished Pharmaceuticals [18] and “cGMP for Phase I Investigational Drugs” [19].

3.2.1.3 Phase II

Phase II clinical studies are usually performed with patients with the condition that is the object of the clinical study. Patient numbers may range from tens to hundreds. Phase II clinical studies can be used to:

- Develop and refine assays
- Perform stability studies
- Begin process validation for removal of impurities
- Evaluate efficacy

Where Phase II studies are used to compare the safety and effectiveness of two or more different doses of the candidate drug, they may be termed “dose ranging” studies. Control measures, such as randomized, placebo usage, and blinded studies, should be employed in order to further validate efficacy studies and prevent bias. Placebo controls are an inactive formulation presented in a way that is indistinguishable from of the candidate drug. Alternatively, another drug known to be effective for the same disease or condition may be used as a comparator to evaluate the relative effectiveness of the new candidate against the previously approved comparator. Blinded studies prevent patients or both patients and the clinical investigators, from knowledge of the use and distributions of the candidate, placebos, and/or comparators in a clinical study. Safety should be evaluated constantly. For further information on comparators, see the ISPE Good Practice Guide: Comparator Management [57].

3.2.1.4 Phase III

Phase III clinical studies are designed to demonstrate the efficacy of the candidate drug in a statistically significant study. Phase III studies are performed with patients with the condition that is the object of the clinical study and may take several years to complete. Patient numbers may range from hundreds to thousands and patients may be located in multiple regions. Phase III clinical studies should evaluate:

- Safety
- Efficacy
- Label indications
- Final release strategies
- Additional studies, such as drug-drug interactions

Key considerations in the design of a Phase III clinical study include the:

- Screening criteria for accepting patients into the study
- Clinical endpoints of the study
- Size of the study (the number of patients)

Placebo controls or a comparator drug should be included in a Phase III clinical study in order to establish statistical significance of the safety and efficacy of the candidate drug. Upon the completion of Phase III clinical studies, the New Drug Application (NDA) for chemical and small peptide product candidates, or the Biologic License Application (BLA) if the product is a biologic, should be filed. The data gathered during the non-clinical and clinical trials of an IND support the NDA. For further information on the regulatory requirements for submission of an NDA, see 21 CFR Part 314, Applications for FDA Approval to Market a New Drug or an Antibiotic Drug [58].

3.2.1.5 Commercialization

Commercialization of a biopharmaceutical drug product typically starts when the decision is made to progress a candidate drug to Phase III clinical studies. The drug sponsor should produce the drug substance and drug product for Phase III clinical studies in the same facilities and using the same process equipment as will be used to produce drug on a commercial scale. Scale-up or process improvements that may be needed to supply the projected commercial markets should be implemented prior to the production of drug substance for Phase III clinical studies. Subsequent changes needed to supply the market should be demonstrated to produce “comparable” drug substance and product through analytical, non-clinical, and/or clinical means

3.2.2 Development Milestones and Associated Considerations

For an overview of the stages of a product profile (with the exception of obsolescence) and considerations for several aspects of product development, see Table 3.1.

Table 3.1: Development Milestones and Associated Considerations

	Pre-IND/Phase I	Phase II	Phase III	BLA/Post Approval
Characterization	Initial	Understanding degradation pathways	Product variants, impact to safety and efficacy (potency)	Complete assessment
Analytical Methods	Specifications based on prior product knowledge and limited manufacturing experience; assays for safety specifications to be validated	Re-evaluate specifications and analytical methods; determine stability-indicating methods and assess as part of validation activities	Re-evaluate specifications and analytical methods	Fully validated analytical methods with justification of acceptance criteria for drug substance and drug product
Process Development	Initial formulation screening; use of platform process; confirmation of viral clearance by manufacturing; viral validation; some flexibility with regards to 21 CFR Part 210 [18] and 21 CFR Part 211 [19]	Process changes, scale up, formulation changes; compliance with 21 CFR Part 210 [18] and 21 CFR Part 211 [19]	Final process optimization; pre-validation activities; DOE studies and QbD; compliance with 21 CFR Part 210 [18] and 21 CFR Part 211 [19]	Validation of drug substance and drug product manufacturing (see FDA Guidance on Process Validation, 2011) [47]; confirm viral clearance on validated process; compliance with 21 CFR Part 210 [18] and 21 CFR Part 211 [19]
Stability	Development studies to support proposed formulation and method development, stability data to cover duration of clinical trials	Forced degradation studies; accelerated stability for comparability as needed	Forced degradation if not completed in Phase II	Complete ICH stability package on commercial product to support expiry dating, shipping parameters and excursions using validated methods
Comparability	Between non-clinical (tox) material and Phase I clinical trial material	As needed to support process and analytical method changes	It is strongly recommended not to make process or assay changes during Phase III. Hold these changes until post-approval. If absolutely required, work with regulatory agencies on a path forward.	Complete assessment of material used in non-clinical and pivotal clinical studies to support safety and efficacy of commercial material
QbD	Quality attributes determined; initial criticality assessment based on prior product knowledge and limited experience with molecule	Process assessment – identify parameters which impact Critical Quality Attributes (CQAs)	Multivariate; DOE studies; risk assessment to determine final control strategy	Continuous process improvement based on extended manufacturing experience; verification of small scale models

3.2.3 **Characterization**

Characterization of biologic products examines the nature of product design and proposed mechanism of action in the context of patient safety and efficacy. Biologics can contain complex heterogeneous mixtures of process and product related impurities and product variants, making characterization more difficult. These attributes and their regulatory implications are discussed.

3.2.4 **Mechanism of Action and Potency**

The proposed mechanism of action of a drug is a major consideration for biologics. Nonclinical studies focus on understanding potential mechanisms of action with the expectation of identifying several potential potency assays that can be used both for lot release and characterization of a drug product, e.g., for monoclonal antibody products, an appropriate binding assay may be sufficient to initiate clinical studies. However, sponsors are expected to develop an assay that provides a representative biological readout prior to initiation of pivotal trials.

There are a few exceptions, e.g., in the case of complex products such as an Antibody-Drug Conjugate (ADC). A biological assay is generally required and a binding assay is not sufficient even for Phase I studies to begin, because of the complex mechanism of action of these products. A pre-IND meeting is recommended for complex products such as these.

The heterogeneous nature of biopharmaceutical products requires characterization of both process and product-related variants, so that manufacturing control strategies can be developed to assure patient safety. Orthogonal analytical methods may be necessary to provide adequate characterization, e.g., in the case of protein aggregates.

Characterization of a biotechnological or biological substance by appropriate orthogonal techniques can be used to gain product knowledge and help to establish relevant specifications. Characterization assays are usually evaluated with regard to the mechanism of action of the molecule and are considered critical to assessing comparability following process changes. The extent of characterization and amount of data usually increase as a product progresses through its life cycle.

Characterization of a biotechnological or biological substance can include the determination of:

- Physico-chemical properties
- Biological activity
- Immuno-chemical properties
- Purity
- Impurities

Supporting evidence to clarify and characterize the product structure should be provided and may include elemental analysis, conformational analysis, and molecular weight determination. Characterization can also include:

- Data on the amino acid sequence
- Peptide map
- Post-translational modifications
- Secondary and tertiary structure information

Initial characterization of a biopharmaceutical product is part of the pre-IND development phase. Assessments should address product safety, purity, and potency. Characterization data gathered during the pre-IND phase should be presented in the original IND submission to provide information on the clarification of the structure of the molecule, its proposed mechanism of action, and known impurities (product and process related).

Examples of product-related impurities include:

- Aggregates
- Fragments
- Charge variants

Examples of process-related impurities:

- Host cell DNA
- Host cell proteins
- Culture and purification components (insulin, anti-foam, residual Protein A, etc.)

Based on characterization, a subset of relevant methods should be chosen for lot release testing of drug substance and drug product. Additional methods also should be selected for in-process monitoring and development of process control strategies.

Detailed characterization studies typically occur during Phase II and include generation of additional information on degradation pathways. Ultimately, characterization should support comparability of batches used throughout development for toxicology and clinical studies with commercial lots.

Data on genetic stability and testing of End of Production Cells should be available at the time of a BLA submission. Data from clearance studies for process related impurities, or a risk assessment strategy justifying why specific impurities do not need to be cleared, should be included in the BLA.

3.2.5 *Analytical Methods*

During the pre-IND phase, analytical methods should be developed as part of IND-enabling activities. Analytical methods fall into three general groups:

1. In-process tests
2. Lot release tests
3. Characterization tests

Some methods, such as those used for sterility and endotoxin, are pharmacopoeial. Other methods, such as those for purity and potency, should be developed to be specific to the molecule and process.

Analytical methods used for lot release should be qualified such that they are suitable for their intended purpose. Typically, analytical methods do not need to be fully validated for Phase I (unless there is a specific safety concern). For Phase II, the suitability of the analytical methods used should be demonstrated. Information should be provided on the validation parameters completed to date (e.g., results or values found for specificity, linearity, range, accuracy, precision, and quantification and detection limits).

When submitting a marketing application, full validation based on ICH Q2(R1) [48] and applicable FDA guidance should be complete for all lot release and stability methods. Validation reports should be included in the submission. This also applies to method validation reports for immunogenicity assays used in non-clinical and clinical studies. Although a full validation is not necessary for pharmacopoeia-based methods, verification that they are fit for their intended use should be demonstrated such that matrix interferences specific to the product do not impact results.

A validated potency assay for inclusion in release and stability testing of both drug substance and drug product is usually required for the start of Phase III (pivotal trials). Validation of all assays is recommended before initiation of Phase III. Samples from toxicology and clinical lots should be retained for assessment in a relevant potency assay to assure consistency among lots.

3.2.6 *Establishing a Process*

Most biologics are temperature sensitive parenteral products that need to be filled under aseptic conditions. Unlike small molecules, the emphasis for development and manufacture of biologics is on the process. A typical outcome from a drug substance manufacturing process is a formulated drug substance that requires no further compounding or mixing and can be directly filled into a glass vial or delivery device (e.g., pre-filled syringe). Interactions of the molecule with the delivery presentation should be understood; however, the actual process development and manufacturing of drug product is usually less complex than development and manufacture of the drug substance.

Throughout development, the biopharmaceutical process may change to meet the required goals for the biopharmaceutical drug product.

The process design can be affected by:

- The type of product desired
- Available biological hosts
- Past biopharmaceutical manufacturing experience of the manufacturing company
- Available manufacturing facilities

Criteria and options considered in defining a biopharmaceutical process may include:

- Criteria to define product (TPP):
 - Potential disease indications
 - Glycosylated versus non-glycosylated protein (can influence choice of host-cell system)
 - Projected market size and global distribution
 - Potential or approved competitors
 - Drug product presentation (IV, SC, autoinjector)
- Process design:
 - Process development life cycle from FIH to long-term commercial support
 - Platform versus tailored approach

- In-process controls based on QbD, including Process Analytical Technology (PAT):
 1. Wide operating ranges
 2. Minimizing of number of CPPs
 3. Robust harvest criteria (wide time window)
- Capacity/scalability considerations
- Upstream facility-fit considerations
 - Batch, Fed-batch, or perfusion production processes
 - Harvest operation requirements
 - Cell culture expansion viable cell density transfer criteria (split ratios)
 - Biosafety level
 - Single-use versus stainless steel
- Downstream facility-fit considerations:
 - Ambient processing
 - Avoid solvents and other hazardous wastes
 - Isocratic elution versus gradient
 - Single-use versus stainless steel
 - Buffer volumes, number of unique buffers:
 1. Cost of buffer raw materials
 2. Flexibility for use of concentrates and stock solutions
 3. Material of construction compatibility (e.g., chloride resistant alloys)
 - Goal of single fraction collection from chromatography steps
 - Column volume, number of cycles per batch, resin cost and lifetime
 - Process integration, minimization of required buffer exchanges
 - Product pool volumes:
 1. Sensitivity of product to concentration dependent aggregation or other degradation
 2. Sensitivity of unit operation performance to product concentration, avoid need for dilute product pools

- Other considerations:
 - Bulk formulation and filling
 - Product quality/patient safety information
 - Optimization, robustness, design space
 - Qualification/validation/reprocessing
 - Regulatory and compliance issues
 - Open versus closed processes
 - Sterile operations versus low bioburden

3.2.7 **Scale-up and Technology Transfer for Commercialization**

A QbD process development program may assist the drug sponsor during scale-up and transfer of the process by establishing a broad design space, which is not facility or equipment specific. QbD can provide a connection between clinical performance and manufacturing process parameters (e.g., CQAs, CPPs). QbD also addresses process variability caused by raw material quality variability and can evaluate interactions between process parameters (multivariate design of experiments approach).

For further information on scale-up and technology transfer for biopharmaceutical products, see Chapter 6 of this Guide.

3.3 **Overview of the Regulatory Implications of Quality by Design and Quality Risk Management**

3.3.1 **Quality by Design**

An enhanced, science- and risk-based approach (i.e., Quality by Design) to biopharmaceutical process development could be considered for the entire process, parts of the process, e.g., unit operations, or parts of a unit operation.

Quality by Design (QbD) is defined in ICH Q8(R2) [6] as:

“A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management.”

The purpose of QbD is to use a systematic approach to develop a high level of understanding about a product and process, and potentially to know the limits of the process and its impact on identified CQAs. The use of a QbD approach also should lead to a more robust process and allow better decisions to be made during development, to help to avoid roadblocks (e.g., re-design of the product or process) or excessive costs. The aim is to design quality into the product and process from the start of development, rather than testing quality into the product at the end of production.

ICH Q11 [9] describes approaches to developing and understanding manufacturing processes of drug substances. Section 3.1.3 of ICH Q11 [9] contains a discussion of the variety of approaches organization could take to development.

ICH Q8(R2) [6] recognizes that:

“Strategies for product development vary from company to company and from product to product. The approach to, and extent of, development can also vary and should be outlined in the submission.”

These concepts apply equally to the development of the drug substance manufacturing process. Pharmaceutical organizations can choose either a traditional approach or an enhanced approach to drug substance development, or a combination of both.

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

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

An enhanced approach to manufacturing process development would additionally include the following elements:

1. *A systematic approach to evaluating, understanding and refining the manufacturing process, including*
 - *Identifying through prior knowledge, experimentation and risk assessment, the material attributes (e.g., of raw materials, starting materials, reagents, solvents, process aids, intermediates) and process parameters that can have an effect on drug substance CQAs*
 - *Determining the functional relationships that link material attributes and process parameters to drug substance CQAs*
2. *Using the enhanced approach in combination with QRM to establish an appropriate control strategy which can, for example, include a proposal for a design space(s).*

The increased knowledge and understanding obtained from taking an enhanced approach could facilitate continual improvement and innovation throughout the product lifecycle (see ICH Q10 [8]).

All biopharmaceutical development programs require identification of a control strategy and CQAs, which are the physical, chemical, biological, or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

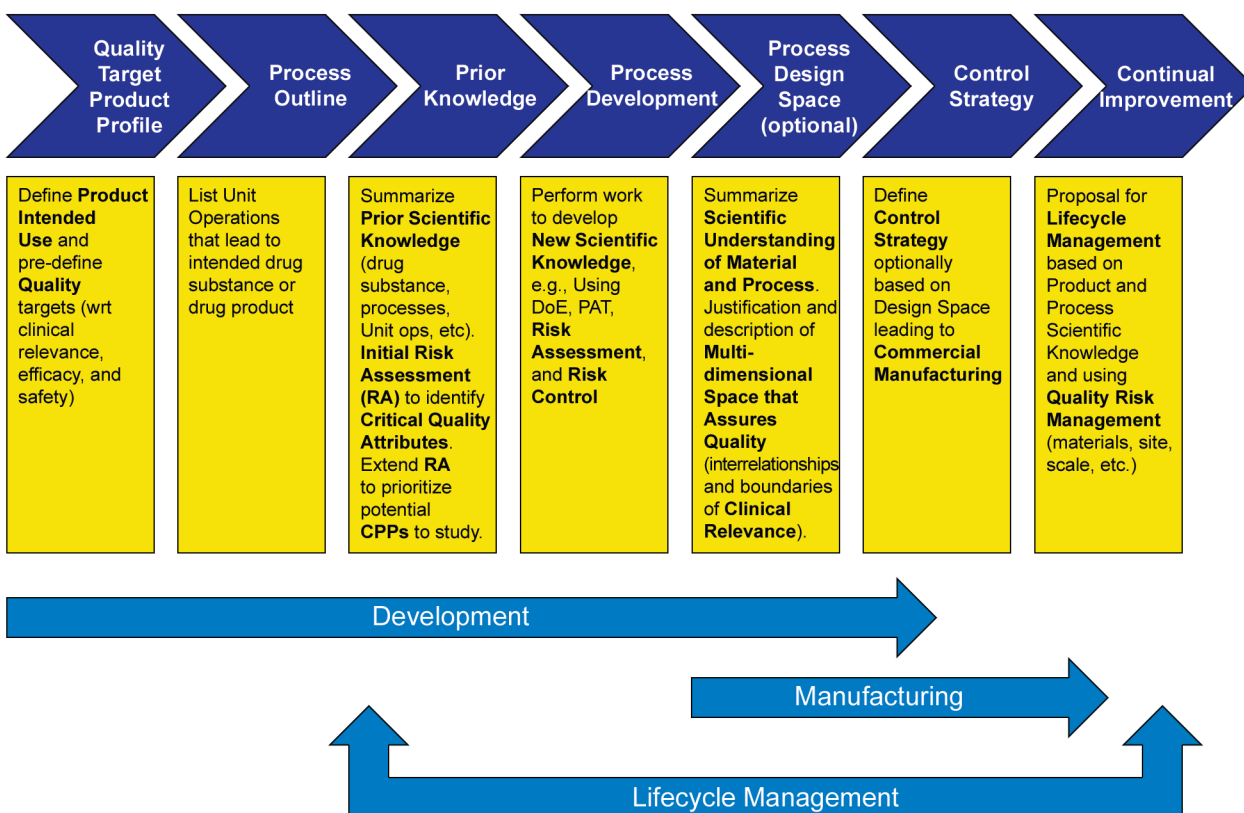
For an overview of QbD approaches to biopharmaceutical process development, see Topic 1, Overview of Product Realization, in the ISPE PQLI® Guide: Part 1 – Product Realization using Quality by Design: Concepts and Principles [59]. This ISPE Guide provides a summary of the benefits of using QbD, which are discussed under the main themes of:

- Making development more efficient
- Improving manufacturing efficiency
- Proposing regulatory flexibility

For further information and examples supporting these benefits, see Kourti and Davis (2012) [60].

The work flow for a QbD approach is given in Topic 1 [59] (Figure 1) and this is based on a schematic developed by a European Federation of Pharmaceutical Industries and Associations (EFPIA) team. Although Figure 3.1 suggests the work flow is linear, there is much discussion and exemplification to indicate that the work is more likely to be iterative, particularly the process development step of sequential and iterative application of quality risk management and design of studies.

Figure 3.1: Product Realization Using Quality by Design



How to assign criticality is discussed, the concept of a continuum of criticality based on risk assessment is suggested with examples. For CQAs, criticality is shown based solely on severity of harm (safety and efficacy) as linked to the patient via the quality target product profile as indicated in the “ICH Points to Consider” document [61]. For CPPs (and critical material attributes), quality risk assessment steps can lead to a continuum of “criticality” based on severity, probability, and detectability.

Summarizing and presenting well these QbD steps and resulting data and conclusions in a regulatory submission should assist in producing an application that is easier for a reviewer to understand and potentially could lead to fewer questions.

3.3.2 The Impact of Quality by Design on Biopharmaceutical Process Development

Biopharmaceutical processes have challenges, which are summarized as:

- Potentially a long list of CQAs, which may have interdependencies
- Potentially many process parameters and material attributes impacting a CQA and one process parameter and material attribute impacting several CQAs
- Lack of basic scientific understanding of some biopharmaceutical processing steps

- Equipment design and scale and facility factors having a significant impact on a CQA

Given these extensive and complex factors, application of quality risk management is extremely important, for example to:

- Rank criticality of CQAs to produce a “continuum”
- Rank importance and hence priority of factors to study
- Rank criticality of process parameters and material attributes
- Choose between control strategy options where choices are available
- Determine appropriate control strategy elements and associated acceptance criteria
- Apply during the process validation life cycle

3.3.3 **Quality Risk Management**

Timely and appropriate application of Quality Risk Management (QRM) can help in gaining knowledge about a product and process. Application of QRM requires specific knowledge of the risk management process and the details of risk assessment tools. The benefits of QRM can be substantial when integrated into an organization's Pharmaceutical Quality System (PQS). Gaps in knowledge can be identified and mitigations implemented more quickly.

The essentials of QRM are provided in ICH Q9 [7], and commented upon further in ICH Q10 [8]. According to ICH Q9, a risk assessment exercise is one part of an overall risk management process. For further information on risk assessments and QRM see Mire-Sluis et al. (2010) [62].

Examples of application of QRM to biopharmaceutical process development are given in the A-Mab Case Study [63]. Throughout this case study, the importance of understanding equipment and facility factors on CQAs is considered and discussed. More specifically, an Ishikawa cause and effect diagram is given in section 3.4.3.2 (of the A-Mab Case Study) relating to production bioreactor and seed culture steps identifying equipment and scale effects. A whole section (3.9) (of the A-Mab Case Study) is devoted to discussing the importance of understanding scale and bioreactor configurations.

From a regulatory perspective, QRM can assist in:

- Justification of specifications
- Proposing and justifying proposed control strategy elements
- Explaining and justifying non-conformances that may lead to unusual circumstances such as reprocessing
- Proposals to adjust an acceptance criterion
- Developing a positive relationship with the regulators (by identifying an established regulatory interaction process for identifying and mitigating risks)
- Determining the type of regulatory submission required to report a given event

3.4 Stability

Stability data in the pre-IND phase should focus on:

1. Preliminary formulation screening exercises
2. IND-enabling stability activities

The stability program should establish the stability of the drug substance and drug product through process or facility changes and periodic market-life stability monitoring.

The stability program should cover the stability of drug substance and drug product throughout biopharmaceutical development and commercial manufacturing. Shelf-life, volume of drug substance and drug product, and cold-chain worldwide distribution logistics should be considered.

Drug substance may need both refrigerated and frozen storage during the product life cycle. The stability program should establish photo-stability and thermostability of the drug substance and drug product. Drug product may encompass multiple dosage sizes and/or presentations including:

- Liquid vials
- Lyophilized vials
- Ampoules
- Pre-filled syringes or autoinjectors

Formulation screening studies should assess stability under the proposed and accelerated storage conditions; these studies are typically short-term. Results from accelerated testing may not be predictive of the degradation pathway and resultant degradants, but the data can provide information on the overall stability of the molecule and/or any key aspects that might need to be monitored or controlled.

Stability data is needed to support initiation of clinical trials. Typically, three months of drug product data (both real time and accelerated) as a minimum is needed to support an initial application. These data could be obtained from an engineering or development lot, as long as the manufacturing processes and release attributes are representative of the material to be used in a clinic. These data may not need to come from the first clinical lot.

Outside of the US, expiry dating is usually required for both drug substance and drug product. The more stability data that are available at the time of submission of the clinical trial application, the easier it will be to supply clinical trials sites and fewer major amendments may be required.

Visible and/or sub-visible particle formation may represent a degradation pathway for monoclonal antibody products and may affect product quality and safety. Testing for visible and sub-visible particles in the accelerated/stressed stability program is recommended to determine whether particulate formation is a component of the degradation pathway. This information may be used to develop and support the testing interval used for particulate testing at the recommended storage temperature.

According to ICH Q1A(R2) [64], stability studies should be conducted on the drug substance packaged in a container closure system that is the same as, or representative of, the packaging proposed for storage and distribution. Materials of construction, particularly the product contact surface, should be identical.

Where non-stainless steel containers are used for storage, a complete analysis of leachables and extractables should be completed for the final container closure system of the drug substance prior to a BLA submission (it is recommended to start earlier, where possible). Shipping studies and agitation studies should be completed prior to a BLA submission. These studies should consider aspects of shipping and include product-specific data. Shipping studies also should include worst-case scenarios for both time and temperature.

4 Upstream Unit Operations

4.1 Cell Line Development

This chapter describes the steps typically used to create the cell line required for production of recombinant proteins. Regulatory requirements should be understood prior to starting cell line development.

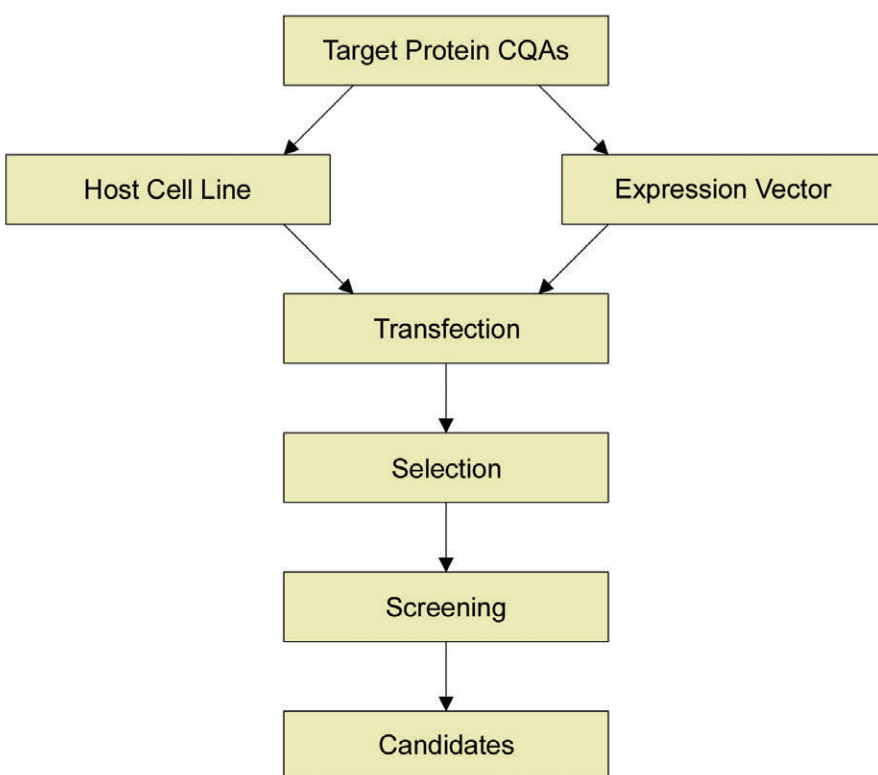
The development of a cell line that expresses the target protein in sufficient quantities to support commercial production is an early step in the development of a recombinant therapeutic protein. The ability to express large amounts of target protein is determined by a combination of:

- Host cell line selection
- Expression vector
- Screening and selection methods

The target protein that is expressed should have the quality attributes required to provide a safe and effective therapeutic action.

Steps used to create cell line candidates are shown in Figure 4.1. The initial step is to identify and understand (to the extent possible during initial development) the potential CQAs of the target protein. The potential CQA information should be used to select the appropriate cell line and expression vector that has the best likelihood of expressing the required target protein. The host cell line and genetic constructs in the expression vector are combined in the transfection step to produce a large number of cell candidates. These candidates should be selected and screened to identify several good candidates for expansion into cell banks.

Figure 4.1: Cell Line Development



Improved cell line development technologies have produced improvements in cell productivity which have resulted in higher upstream production yields. Platform cell line development technologies can reduce the development time and work required to support regulatory submissions and assist the regulatory approval process. These technologies should be screened and evaluated prior to initiating a cell line development program.

Relevant regulatory guidelines should be read and understood prior to initiation of cell line creation activities.

Cell lines should be developed from a well characterized host using media free from animal-derived components or infectious materials. When creating cell lines, manipulations should be performed using appropriate Good Manufacturing Practices (GMPs). Laboratory testing, including contract testing, should be conducted according to Good Laboratory Practices (GLPs). This may differ for other jurisdictions, such as the EU and Japan. Good practices also include the use of appropriate facilities with a controlled environment using biosafety cabinets, and aseptic techniques by qualified, suitably trained, and experienced personnel. Cell line development should be documented according to Standard Operating Procedures (SOPs) to produce detailed manufacturing records and batch records suitable for inclusion in regulatory submissions.

4.1.1 Target Protein Attributes

The first step in establishing a cell line is to understand the attributes of the target protein. The target protein should be characterized in regard to the potential CQAs required to be safe and effective in the therapeutic application, as far as is practical. Potential CQAs may include:

- Primary structure (amino acid sequence)
- Secondary, tertiary, and quaternary structural elements (if present)
- Glycosylation
- Disulfide bonds

Potential CQAs should be included in a preliminary draft of a Quality Target Product Profile (QTPP) (see in ICH Q8(R2) [6]) which should be used to select the expression vectors and possible host cell candidates. Defining the required CQAs may involve significant research and be combined with expert medical advice, but the preliminary draft QTPP can be used to assist in identifying the initial genetic construct and host cell line. For further information on the CQAs that need to be considered when selecting a host cell line and expression vector, see ICH Q6B [4].

4.1.2 Host Cell Line Selection

Options for host cell systems include:

- Bacterial cell lines
- Mammalian cell lines
- Yeast cell lines
- Insect cell lines
- Plant cell lines

This Guide considers the development of bacterial and mammalian host cell lines although many of the principles may be applied to the development of other types of cell line development.

Advantages and disadvantages of host cell systems should be evaluated according to the following criteria. Selection of an appropriate host cell line is determined by three factors, for which the cell line needs to:

1. Be capable of producing a biologically active protein with the appropriate CQAs required for safety, efficacy, and potency
2. Be capable of growing at high densities in the appropriate bioreactor system while expressing the target protein at a high level, to provide sufficient production capacity to support commercial manufacturing
3. Have a well-defined, documented history to meet various regulatory requirements

Identifying a host cell line that can produce a safe and effective target protein that meets the CQAs described in of the QTPP may be difficult. The QTPP and its impact on host cell line selection should be understood. For example, the cell may need to be able to provide required post-translational processing to make the target protein safe and biologically active. Where the post-translational CQA specifies that the target protein must be glycosylated, the use of prokaryote organisms (e.g., *E.coli*) would be eliminated as they are not capable of glycosylating proteins. Eukaryotes, particularly mammalian cell lines (e.g., Chinese Hamster Ovary (CHO)), can be used to produce a wide variety of glycosylation. As analytical methods continue to evolve, more CQAs are being identified making the selection of appropriate QTPP elements more difficult.

Following identification of product CQAs, an appropriate cell line and bioreactor operating scheme should be determined. Estimates of target protein requirements based on dose and market size should be assembled in order to establish likely production capacity requirements. Bioreactor options should be evaluated based on cell line options to meet anticipated capacity requirements. Options range from very larger bacterial fermentations to small scale mammalian cell run in hollow fiber perfusion reactions. Cell line-bioreactor combinations should be able to satisfy QTPP-capacity requirements.

A host cell line should be derived from a known source with appropriate documentation detailing where it was originally obtained and a complete history, including all manipulations and modifications. Standard cell lines are usually available from tissue banks and other known sources. Suppliers may have standard cell lines which are part of a platform technology for manufacturing recombinant proteins. The use of a platform technology can provide a well-documented host cell line with known protein expression capabilities and production capacity experience. The platform technologies also may provide a variety of cost and time saving advantages. Such technologies also may provide guidance on the next step to achieving the production of recombinant proteins, i.e., the appropriate expression vector.

4.1.3 Expression Vector

The expression vector includes the DNA sequence (cDNA) required to make the target protein's amino acid sequence (primary structure). However, a number of other nucleotide sequences are also added to facilitate expression of the target protein and assist with screening cell candidates. Elements of the expression vector include the promoter and enhancer sequences, along with antibiotic resistance genes used to screen the cell populations.

For further information, see ICH Q5B "Analysis of the Expression Constructs in Cells Used for Production of r-DNA Derived Protein Products" [3] and the FDA's "Supplement to Points to Consider in the Production and Testing of New Drugs and Biologics Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability" (1992) [65].

There can be a wide variety of expression systems along with several vector design strategies. Assembly of the appropriate expression vector is complex and requires significant expertise. Vector components are carefully constructed in a laboratory prior to the transfection step. Sources of all genetic sequences should be identified and documented, along with the methods used to assemble and manipulate the genetic material. For some fermentation strategies, an induction sequence may be added to facilitate the controlled start of target protein manufacturing (induction) by shifting the cell's metabolic pathways.

Expression vector options may be covered by proprietary technologies and the Intellectual Property (IP) implications of a selected vector system should be understood. Expression vector platform technologies from suppliers may be used to help to secure an acceptable IP position. Where needed, licenses should be obtained prior to the start of development activities.

4.1.4 **Transfection**

After the host cell candidate is selected and the expression vector assembled, the two are combined in order to insert the genetic material construct into the host cell. This process is usually called transfection in mammalian cells and may be called transformation in bacterial cells systems although the terminology varies from organization to organization. This Guide uses the term **transfection**. After the genetic material, or plasmid, enters the cell, it is incorporated into the cell's genetic structure as translatable genetic material within the cell.

The genetic construct can be integrated into the cell in two ways:

1. Extrachromosomal or transient integration places the genetic construct in a plasmid configuration which the cell will use to express the target protein along with other protein elements. Inserting a plasmid, or self-contained closed set of genetic material, is a form of extrachromosomal integration.
2. Intragenome integration occurs when the genetic construct is incorporated directly into the chromosomes of the cell genome. In this case, the target protein is expressed along with other cell proteins as part of the cells normal protein expression system.

Intragenome integration is considered to be more stable than extrachromosomal integration.

Transfection, or the opening of the cell wall, can be accomplished using physical and chemical methods. Calcium phosphate co-precipitation in conjunction with other additives can be used. Electroporation uses a pulsed electric shock to open the cell wall and allow the external genetic material to enter. Several other transfection methods, e.g., microinjection, lipofection, and polyfection can be used depending on the particular requirements of the host cell line.

After the cell wall is opened and the genetic material introduced, the cell is allowed to reform its cell wall. Typical efficiencies are below 25% and many cells are unable to recover from the transfection process.

Once the cell wall has been made porous, the genetic material is free to enter the cell's genetic structure. After transfection, a subpopulation of the cells recover and can be screened to identify a cell population that expresses target protein.

Incorporation of the genetic material within the cell is a random process where the genetic modifications may occur in a wide variety of locations. In a majority of the cells, the incorporation does not produce a cell that will express the target protein. The initial screening process utilizing the antibiotic resistance genes included in the expression vector are used to identify the cells which have been appropriately modified. The next step is to find the cells that express the correct target protein.

4.1.5 **Selection of Candidates**

Cells may be killed by the transfection process. For the surviving cells, the expression vector includes genes that impart antibiotic resistance to the cells which have been successfully transfected. The selection media contains antibiotic which inhibits the growth of cells which have not been transfected. Other cell selections tools exist, but these are outside the scope of this Guide; for further information, Thalhamer, Weiss, and Scheibelhofer (2012) [66] and Perry (2007) [67].

Following selection and expansion into dilute culture, monoclonal populations are identified and separated. The initial screen is usually for high productivity. Cells are further screened for: