

The People's Democratic Republic of
Algeria
Ministry of Higher Education and
Scientific Research
Ferhat Abbas University Sétif 1
Faculty of Nature and Life Sciences



الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي
جامعة فرحات عباس سطيف 1-
كلية علوم الطبيعة و الحياة

Biotechnology Department

Course Handout in :

Control and Monitoring Techniques for the Manufacturing Process of a Biopharmaceutical

Level: 1st Year Master's Degree (LMD) in Biotechnology and Health

Prepared by: **Dr. Karima LOUCIF**

Academic Year: 2025/2026

Course content

Introduction	1
Chapter I	2
I. Biopharmaceutical Manufacturing Process	2
1. Manufacturing Process of Allopathic Medicines	2
1.1 Definition of Allopathic Medicines	2
1.2 Therapeutic Models of Allopathic (Homeopathic) Medicines Compared to Homeopathic Medicines	2
1.3. Characteristics of Allopathic Medicines	4
1.4. Contemporary Classification of Allopathic Medicines and the Integration Between Chemical Molecules and Biological Innovations	5
1.5. Chemical Drugs Manufacturing Process.....	5
1.5.1. Active Pharmaceutical Ingredient (API) Design.....	5
1.5.2. Raw Materials Procurement.....	6
1.5.3. Chemical Synthesis of the Active Pharmaceutical Ingredient (API)	6
1.5.4. Monitoring of the Chemical Reaction	7
1.5.4.1. Parameters Monitored During the Reaction.....	8
1.5.4.2. Methods for Monitoring Reaction Progress.....	9
1.5.4.3. Control of Reaction Conditions During Monitoring	10
1.5.4.4. Determination of Reaction End Point	11
1.5.5. Separation and Purification of the Active Substance	11
1.5.6. Quality Control of Active Pharmaceutical Ingredient (API)	13
1.5.7. Pharmaceutical Formulation	13
1.5.8. Packaging.....	14
1.5.9. Storage and Distribution	16
2. Biologic Drugs Manufacturing Process	16
2.1. Gene and Cell Line Selection	17
2.2. Cell Lines and Cell Banks.....	19
2.3. Bioreactor Cell Culture and Recombinant Protein Expression (Upstream Processing)	22
2.4. Separation and Purification of Recombinant Protein (Downstream Processing)	23
2.5. Quality Control and Bioassays of Recombinant Protein	23
2.6. Final Drug Formulation.....	24
2.7. Filling and Packaging	24

Course content

2.8. Storage and Distribution	25
3. Key Differences Between Biologic Drugs and Chemical Drugs	27
4. Uses of Biological Drugs Compared to Chemical Drugs.....	28
5. Conclusion.....	31
Chapter II.....	32
II. Quality Control in Biopharmaceutical Manufacturing	32
1. Physicochemical Control Techniques.....	32
1.1 Physical Control Techniques.....	33
1.2 Chemical Control Techniques.....	34
2. Microbiological Control Techniques	36
2.1 Concept of Microbiological Control	36
2.2 Importance of Microbiological Control in Biopharmaceutical Manufacturing	36
2.3 Sources of Microbial Contamination.....	37
2.4. Monitoring of Potential Sources of Microbial Contamination	37
2.4.1. Raw Material Monitoring	37
2.4.2. Monitoring of Manufacturing Environment.....	38
2.4.2. Manufacturing Environment Monitoring	39
2.4.3. Personnel and Equipment Monitoring.....	40
2.4.4. In-Process Monitoring and Verification of Bioburden Control.....	41
2.4.5. Supportive Safety Monitoring	42
2.4.5.1. Aseptic Filling Simulation Monitoring.....	42
2.4.5.2. Preservative Effectiveness Monitoring Preservative Effectiveness Test – PET	42
2.4.6. Final Product Monitoring and Testing.....	43
2.5. Microbiological Tests Used in the Monitoring of Biological Drug Manufacturing	44
2.5.1. Bioburden Test	45
2.5.2. Microbial Enumeration Tests	45
2.5.2.1. Total Aerobic Microbial Count (TAMC).....	46
2.5.2.2. Total Yeast and Mold Count (TYMC)	46
2.5.2.3. Total Viable Count (TVC).....	46
2.5.2.4. Most Probable Number (MPN / NPP).....	47
2.5.3. Sterility Test	47
2.5.3.1. Membrane Filtration Method	47
2.5.3.2. Direct Inoculation Method	48
2.5.4. Specified Microorganisms Test.....	48

Course content

2. 5. 5. Anaerobic Bacteria Detection Test.....	50
2. 5. 6. Bacterial Endotoxin Test.....	51
2. 5. 7. Mycoplasma Detection Test.....	52
2. 5. 8. Viral Detection Test.....	52
2. 5. 9. Rapid Microbiological Methods (RMMs).....	54
2. 6. Linking Test Results to Manufacturing Decisions.....	55
2. 6. 1. International Regulatory Systems and Authorities.....	55
2. 6. 1. 1. Quality Systems / Quality Standards.....	56
2. 6. 1. 2. Regulatory Authorities / Organizations.....	56
2. 6. 3. Key Acceptance Criteria in Biological Drugs.....	57
2. 6. 4. Manufacturing Decision Making.....	58
3. Toxicity Monitoring Techniques.....	59
3.1. Definition of Toxicity Monitoring Techniques.....	60
3.2. Importance of Toxicity Monitoring Techniques.....	61
3.3. Types of Toxicity in Biopharmaceuticals.....	61
3.3.1. Cytotoxicity.....	62
3.3.2. Immunotoxicity.....	63
3.3.3. Systemic Toxicity.....	64
3.4. <i>In vitro</i> & <i>In vivo</i> Toxicity Assays.....	66
3.5. Toxicity Evaluation Parameters.....	67
Conclusion.....	69
Chapter III.....	70
III. Immunostaining and Immunodetection Techniques.....	70
1. Definition of Immunostaining and Immunodetection Techniques.....	70
2. Importance of Immunostaining and Immunodetection Techniques in Biopharmaceutical Manufacturing.....	71
3. Principle of Antigen–Antibody Reaction.....	71
4. Immunostaining and Immunodetection Techniques Used in Biopharmaceutical Monitoring.....	72
4.1. Immunostaining Techniques.....	73
4.1.1. Immunofluorescence.....	73
4. 1. 2. Immunohistochemistry.....	75
4. 2. Immunodetection Techniques.....	76
4. 2. 1. Enzyme-Linked Immunosorbent Assay (ELISA).....	76
4. 2. 2. Western Blot.....	78

Course content

4. 2. 3. Flow Cytometry-based Immunodetection	80
5. Applications of Immunological Techniques in Biopharmaceuticals	82
6. Advantages and Limitations of Immunological Techniques	82
6.1 Advantages of Immunological Techniques.....	82
6.2 Limitations of Immunological Techniques.....	83
7. Conclusion	83
General Conclusion	85
Bibliography	88

Introduction

Introduction

Biopharmaceutical manufacturing process monitoring and control techniques constitute pivotal approaches in the field of health biotechnology, due to their essential role in ensuring the quality and safety of modern pharmaceutical products, particularly biopharmaceuticals (Biomédicaments), which represent one of the most significant advances in the contemporary pharmaceutical industry. This importance is further reinforced by their growing role in the treatment of complex and chronic diseases, as well as their reliance on biological systems or bioengineering technologies in production, in addition to the stringent requirements and high technical complexity across the different stages of manufacturing, owing to their sensitivity and the structural complexity that distinguishes them from conventional drugs.

The importance of biopharmaceutical manufacturing process monitoring and control techniques lies in their role as a structured and integrated system aimed at ensuring the quality, safety, and efficacy of pharmaceutical products, starting from raw materials, progressing through the various production stages, and extending to the final product as well as storage and distribution conditions. This system encompasses multiple levels of analysis and control, including physicochemical analyses, microbiological testing, and toxicological evaluations, in addition to quality assurance mechanisms implemented throughout all manufacturing stages. Immunolabeling and immunodetection techniques (Immunomarquage et immunodétection) are particularly important in this context, as they provide high-precision tools for the analysis, characterization, and tracking of biomolecules within biological systems, thereby strengthening monitoring and quality control of biological products.

Quality assurance in the modern pharmaceutical industry is fundamentally based on the implementation of international standards and regulatory frameworks, particularly Good Manufacturing Practices (GMP) and Good Laboratory Practices (GLP), in addition to Hazard Analysis and Critical Control Points (HACCP) systems, which collectively ensure compliance of pharmaceutical products with health and regulatory requirements.

Chapter I

I. Biopharmaceutical Manufacturing Process

1. Manufacturing Process of Allopathic Medicines

1.1 Definition of Allopathic Medicines

The term “Allopathy” was coined by the German physician Samuel Hahnemann at the beginning of the nineteenth century (around 1810) to distinguish the conventional, official, and systematic medical system (not his own system), which was dominant at that time, from his then-innovative system known as Homeopathy. The term “Allopathy” is composed of two Greek words: “Allo,” meaning “different,” “other,” or “opposite,” and “Pathos,” meaning “suffering” or “disease,” which reflects the principle of treatment by opposites: allopathic treatment produces an effect that counteracts the symptoms of the disease. In the contemporary academic context, “allopathic medicines” refer to Evidence-Based Medicine. These are drugs (whether of chemical molecular origin or biological origin) that undergo strict research protocols and clinical trials to demonstrate their efficacy and safety before pharmaceutical approval. In contrast, “treatment by similars” (Homeopathy) is a branch of alternative medicine, non-systematic and non-official (alternative medicine, also called parallel medicine, includes all practices outside the scope of official medicine, such as treatment by similars or homeopathic treatment, herbal medicine, acupuncture, yoga, massage, dietary supplements, and vitamins). It is based on the idea that a substance that causes certain symptoms in a healthy person can, in highly diluted form, be used to treat a patient suffering from the same symptoms.

1.2 Therapeutic Models of Allopathic (Homeopathic) Medicines Compared to Homeopathic Medicines

➤ **Opposite-treatment principle:**

- If a patient suffers from “fever” (elevated body temperature), a drug is administered that acts to “reduce temperature.”
- If a patient suffers from a “bacterial infection,” an “antibiotic” is given to eliminate the causative agent.

➤ **Hahnemann's principle of "like cures like":**

- The drug "Allium Cepa" (derived from red onion) is used to treat patients suffering from acute colds or allergic rhinitis (Hay Fever), which present symptoms identical to those caused by cutting onions (runny nose and watery eyes). Similarly, "Arsenicum Album," a highly toxic mineral that in large doses causes severe gastric burning, vomiting, watery diarrhea, and acute anxiety with fear of death, is used in highly diluted doses to treat food poisoning cases presenting the same symptoms (burning abdominal pain, diarrhea, and severe psychological anxiety).
- Ingestion of large quantities of table salt (Natrium Muriaticum) leads to dehydration, migraine, and dry skin; in homeopathic treatment, it is used in diluted doses to treat chronic migraines and dry skin conditions.
- Bee venom (Apis Mellifica) is used to treat swollen joint inflammations, urticaria (Hives), or even sore throat, provided that the symptoms resemble a bee sting reaction (pink swelling, stinging pain, and relief with cold applications).
- The venom of the snake "Lachesis mutus" affects the circulatory system and can cause bleeding, a sensation of suffocation, or tightness when the neck is touched. In homeopathy, it is used to treat menopausal disorders (hot flashes) or tonsillitis predominantly affecting the left side, especially when the patient experiences severe tightness in the neck.
- For insomnia, Hahnemann may prescribe a highly diluted dose of "Coffee" (Coffea Cruda), since it causes insomnia in healthy individuals; therefore, it is used to treat the same condition in patients. In contrast, in allopathic medicine, a patient with insomnia is given a "sedative" (a substance that produces an effect opposite to wakefulness), which reflects the "opposite-treatment" principle.

➤ **Dietary supplements:**

- Dietary supplements and vitamins are classified as shared therapeutic tools; they are considered allopathic when used to correct a scientifically proven physiological

deficiency, such as prescribing “vitamin D” for a patient with a confirmed severe deficiency based on laboratory tests, or “iron” for a patient with anemia. In this case, they are treated as “allopathic drugs” because they are administered to correct a scientifically confirmed biochemical functional deficit in precise doses.

- They are considered complementary/alternative medicine when used to enhance vitality or within non-systematic protocols, such as a healthy individual taking high doses of vitamins to “boost energy” or “detoxify” without any laboratory-confirmed deficiency, or using them to treat severe diseases (such as cancer) based on theories not fully validated in allopathic clinical trials.

1.3. Characteristics of Allopathic Medicines

These drugs are characterized by several features that make them an essential part of modern medicine compared to alternative medicine:

- Scientific evidence-based approach: They undergo strict clinical trials (Clinical Trials) to demonstrate their efficacy and safety.
- Direct targeting: They mainly focus on treating the affected organ or the direct cause of the disease (such as viruses or bacteria).
- Speed: They are generally faster in relieving acute and emergency symptoms compared to alternative medicine.
- Chemical composition: They primarily rely on chemically synthesized compounds or laboratory-processed extracts at very specific concentrations.
- Most traditional and modern allopathic drugs are “chemical drugs,” while biopharmaceuticals represent part of modern allopathic medicines.
- Practical example: When a doctor prescribes a drug for hypertension (chemical) or an insulin injection (biological), in both cases this is considered “allopathic medicine,” because the treatment targets a pathological symptom using a substance that produces an opposing effect.

1.4. Contemporary Classification of Allopathic Medicines and the Integration Between Chemical Molecules and Biological Innovations

Medical practice has evolved from a historical duality based on “traditional medicine”, inherited practices such as herbal medicine and cupping therapy, and “old allopathic medicine,” which relied on simple chemical drugs, to a modern system (Conventional Medicine) structured into two methodological pathways. The first is “contemporary allopathic medicine,” the official evidence-based system in which “chemical drugs or synthetic chemical molecules” constitute the vast majority of available therapeutic options and global pharmaceutical production, with a qualitative expansion that now includes high-technology biological medicines. The second is “alternative medicine,” which includes traditional folk medicine and all non-systematic therapeutic practices that fall outside official academic and regulatory frameworks.

1.5. Chemical Drugs Manufacturing Process

Chemical drugs are defined as pharmaceutical preparations obtained through specific chemical reactions between relatively simple starting materials, where the final active compound has a precisely known molecular formula, structure, and molecular weight. These drugs are characterized by the possibility of manufacturing them through relatively standardized steps, with easier control over their purity and composition compared to biological drugs. The manufacturing process of chemical drugs begins with the selection of the active ingredient and ends with obtaining the final pharmaceutical dosage form ready for marketing. Although details vary depending on the nature of the drug, the general stages remain similar across most chemical pharmaceutical industries.

1.5.1. Active Pharmaceutical Ingredient (API) Design

In the first stage, the pharmacologically active substance is identified, i.e., the compound responsible for the therapeutic effect. This is usually achieved through a series of chemical and pharmacological studies aimed at: determining the therapeutic target, identifying the receptor or enzyme with which the drug will interact, selecting the most effective chemical structure, and optimizing the compound’s properties such as solubility, stability, and absorption.

The active substance may be discovered through two main approaches:

1. Extraction from natural compounds followed by chemical modification.
2. Full chemical synthesis in the laboratory based on synthetic chemistry.

Example: many antibiotics or anti-inflammatory drugs were developed from initial compounds that were chemically modified to improve efficacy and reduce toxicity.

1.5.2. Raw Materials Procurement

After selecting the active substance, the necessary raw materials for production are supplied, including reagents, solvents, catalysts, and excipients. These materials must comply with pharmaceutical standards in terms of purity, moisture level, absence of metallic or microbial impurities, and storage stability. Before any raw material is approved for use in the manufacturing facility, quality control tests are performed, including identification, purity testing, moisture content measurement, and impurity profile analysis.

1.5.3. Chemical Synthesis of the Active Pharmaceutical Ingredient (API)

This stage represents the main step in the manufacturing process, where raw materials are converted into the final active substance through a series of sequential chemical reactions. These reactions may include oxidation, reduction, esterification, hydrolysis, substitution, and condensation. These reactions are carried out in chemical reactors equipped with systems for controlling temperature, pressure, stirring speed, pH value, and reaction time. Controlling these parameters is essential, as any slight variation may lead to reduced reaction yield, formation of unwanted impurities, alteration of the molecular structure, or loss of therapeutic efficacy. In some cases, the synthesis of the active ingredient requires multiple successive steps, where each stage produces an intermediate Product that is used in the next stage until the final product is obtained.

Simplified example:

$A + B \rightarrow C$ (intermediate compound)

Then: $C + D \rightarrow$ final API product.



Figure 01: Chemical reactor inside a pharmaceutical manufacturing plant.

1.5.4. Monitoring of the Chemical Reaction

The monitoring of the chemical reaction is considered a critical stage in the manufacturing of the active pharmaceutical ingredient. It is not sufficient to mix the starting materials and allow the reaction to proceed until completion; instead, all changes occurring during the reaction must be continuously or periodically monitored to ensure that the desired compound is correctly formed and that the reaction proceeds toward the target product with maximum yield and purity.

This stage aims to:

- Ensure the gradual consumption of reactants.
- Determine the appropriate time to stop the reaction.
- Early detection of by-product formation or impurities.
- Adjust reaction conditions when necessary.
- Increase the yield of the active substance and reduce losses.

This stage is particularly important in the pharmaceutical industry, as extending the reaction time beyond the required duration or stopping it prematurely may lead to a reduction in the amount of active substance or the formation of impurities that may be toxic or difficult to remove during the purification stage.

1.5.4.1. Parameters Monitored During the Reaction

During the chemical reaction, a set of physical and chemical parameters is monitored to evaluate reaction progress. The most important parameters include:

- **Temperature**

Temperature is one of the most important factors affecting reaction rate and direction. An increase in temperature may accelerate the reaction but may also lead to degradation of the active substance or the formation of unwanted by-products, while excessively low temperatures may slow down the reaction or prevent its completion. Therefore, temperature is continuously monitored using thermal sensors connected to an automated control system that maintains it within a predefined range.

- **pH**

In some reactions, particularly those involving acids, bases, or sensitive compounds, changes in pH serve as a direct indicator of reaction progress. Even slight variations in pH may alter the nature of the formed product or lead to degradation of the active substance.

- **Reaction Time**

Each reaction has an optimal time required to achieve maximum yield. Therefore, samples are taken at regular intervals to assess reaction progress and determine when the reaction reaches completion.

- **Reactants and Products Concentration**

The concentrations of starting materials and products are regularly measured to determine the conversion rate of reactants into the active substance. A decrease in reactant concentration and stabilization of product concentration generally indicate reaction completion.

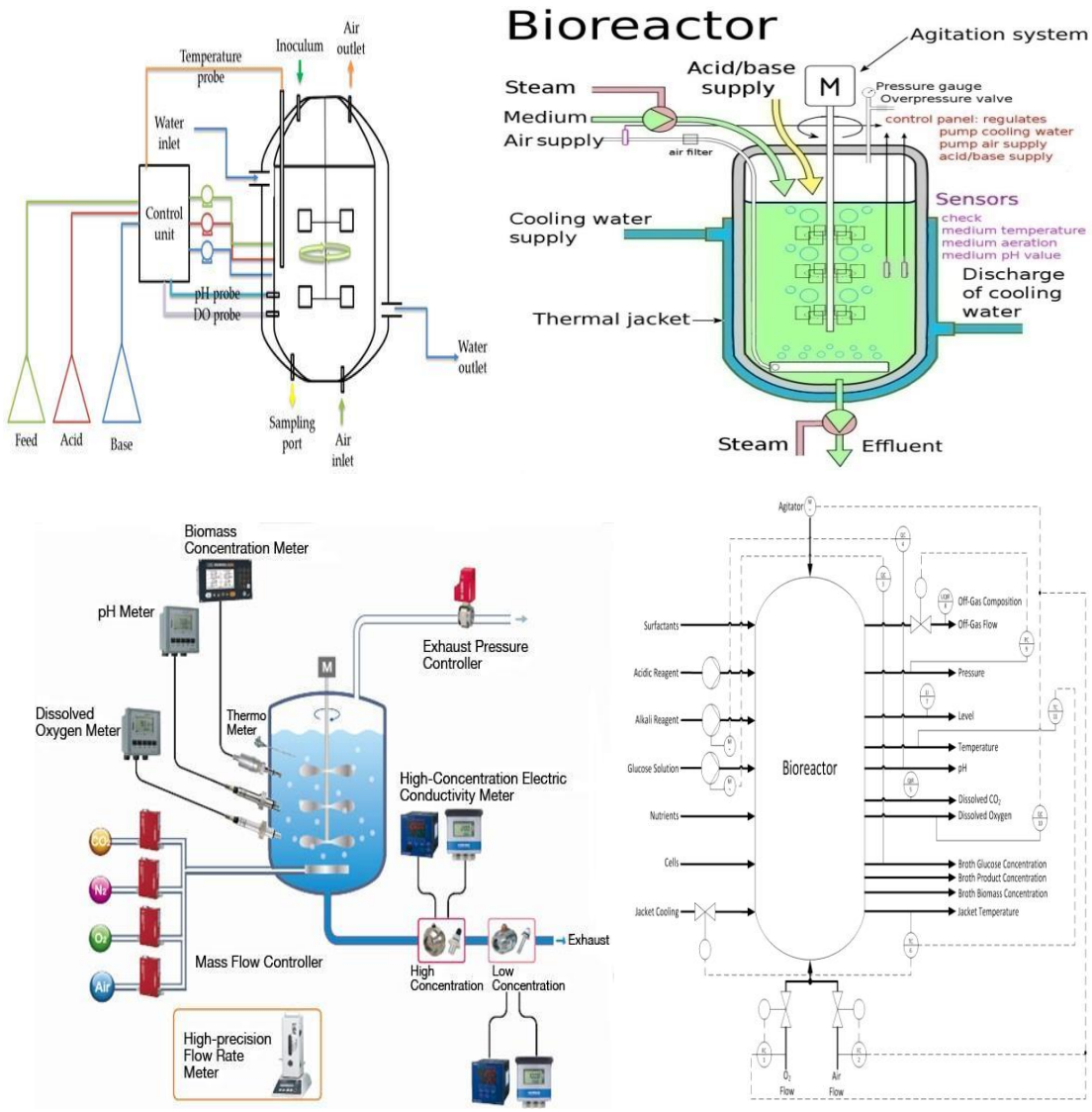


Figure 02: Control of bioprocess parameters inside bioreactors.

1.5.4.2. Methods for Monitoring Reaction Progress

Visual observation alone is not sufficient; instead, precise analytical techniques are used to detect the substances present within the reaction mixture.

• Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is one of the simplest and most widely used techniques in laboratories. It is based on comparing the movement of reactants and the final product on a plate coated with a thin layer of silica. At the beginning of the reaction, a spot representing the starting material is observed. This spot gradually decreases over time while a

new spot representing the product appears. When the spot of the starting material almost disappears, the reaction is considered complete.

$$R_f = \text{Distance traveled by compound} / \text{Distance traveled by solvent front}$$

The R_f value represents the distance traveled by a compound relative to the solvent front and is used to distinguish different substances during the reaction.

• High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC) is used when higher precision is required, particularly in the pharmaceutical industry. This technique allows accurate quantification of the active substance, starting materials, and impurities. Therefore, it is mainly used in industrial stages rather than only in laboratory settings.

• Infrared Spectroscopy (IR)

Infrared (IR) spectroscopy is used to monitor the disappearance or appearance of functional groups during the reaction. For example, if the starting material contains a hydroxyl group and this group disappears in the final product, the disappearance of its characteristic IR signal indicates that the reaction has occurred.

• Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) is particularly used when the reaction is complex or when there is a possibility of forming multiple products. It allows determination of the precise chemical structure of the product formed during the reaction.

1.5.4.3. Control of Reaction Conditions During Monitoring

Monitoring is not limited to measurement; it may also lead to adjustments in reaction conditions, such as:

- Increasing or decreasing temperature.
- Adding additional amounts of reactants.
- Changing stirring speed.
- Adjusting pH value.
- Adding a solvent or catalyst.

These adjustments aim to direct the reaction toward maximum yield of the active substance with minimum impurity formation.

1.5.4.4. Determination of Reaction End Point

After monitoring all previous parameters, the moment at which the reaction is stopped must be identified. This moment is known as the reaction end point.

The reaction is considered complete when:

- Most starting materials have been consumed.
- The product concentration becomes stable.
- No further changes in temperature or pH are observed.
- No new impurities are detected during analysis.

Stopping the reaction at the correct time prevents degradation of the active substance or its conversion into secondary products that may reduce drug quality.

1.5.5. Separation and Purification of the Active Substance

After completion of the chemical reaction, the resulting product is not completely pure. It often contains unreacted starting materials, reaction by-products, residual solvents, and impurities. Therefore, multiple separation and purification processes are carried out to obtain a high-purity active pharmaceutical ingredient. The main techniques include filtration, crystallization, extraction, distillation, and chromatography. Crystallization is one of the most widely used methods, as it allows the production of relatively pure crystals of the active substance.

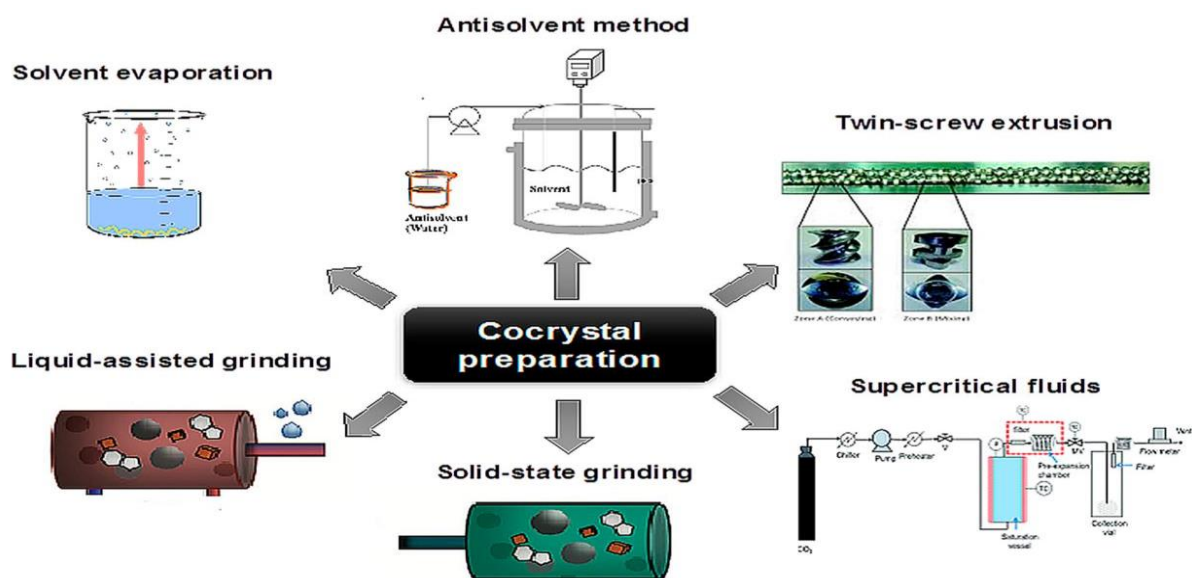
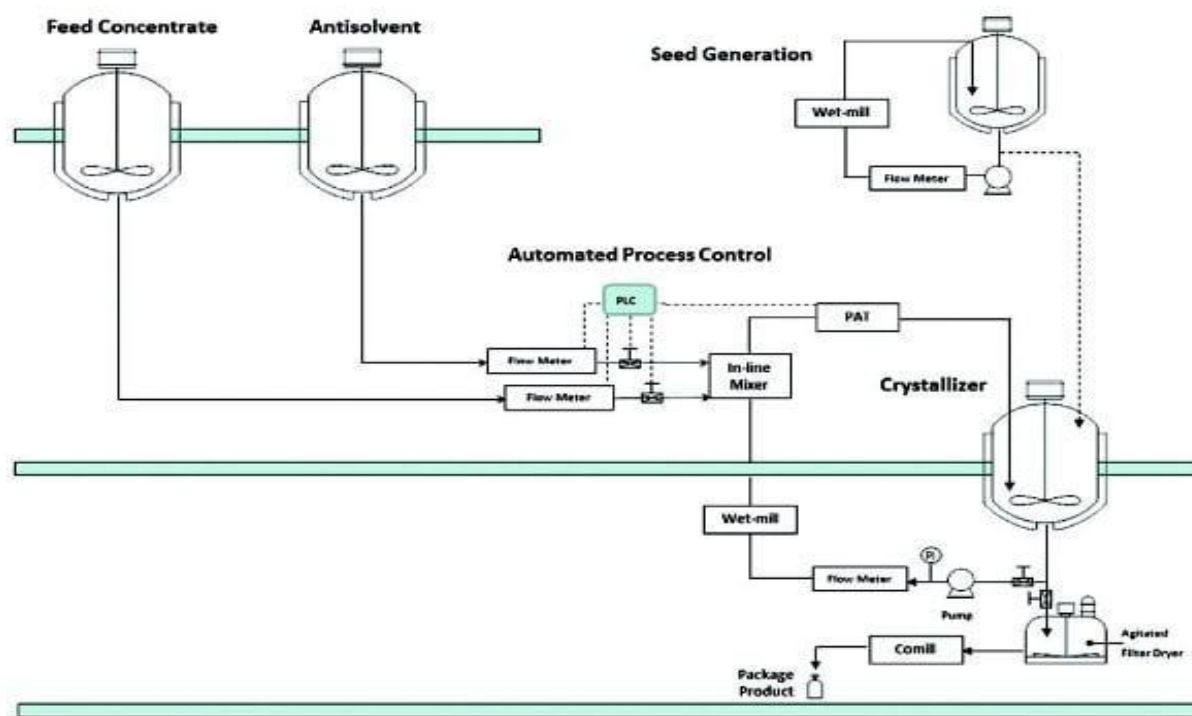


Figure 03: The image illustrates the separation of the active pharmaceutical ingredient after the crystallization stage and upon completion of the chemical reaction.

1.5.6. Quality Control of Active Pharmaceutical Ingredient (API)

Before using the Active Pharmaceutical Ingredient (API) in the preparation of the pharmaceutical dosage form, it must be ensured that it complies with all pharmaceutical specifications. Quality control tests usually include: Identification Test, Purity, Assay, detection of organic and inorganic impurities, Residual Solvents, and Stability Testing.

Among the most commonly used techniques in this stage: High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Infrared Spectroscopy (IR), Ultraviolet-Visible Spectroscopy (UV-Vis), and Mass Spectrometry (MS). These techniques are used to ensure that the chemical structure of the compound is correct and that its concentration is within the required limits.



Figure 04: HPLC instrument used in the quality control of chemical drugs.

1.5.7. Pharmaceutical Formulation

After confirming the quality of the Active Pharmaceutical Ingredient (API), it is mixed with excipients to obtain the appropriate pharmaceutical dosage form, such as: Tablets, Capsules, Solutions, Ointments, and Injections. Excipients play different roles, such as improving taste, increasing stability, facilitating absorption, and providing the appropriate final dosage form. For example, a pharmaceutical tablet usually contains: Active pharmaceutical ingredient, Filler, Binder, Disintegrant, and Lubricant. After mixing, the preparations undergo several industrial processes such as Granulation, Drying, Compression, and Coating.



Figure 05: Tablet compression machine inside the manufacturing facility.

1.5.8. Packaging

After the completion of the manufacturing of the pharmaceutical dosage form, it is packaged into suitable containers that maintain its stability and protect it from moisture, light, oxygen, and contamination.

Packaging types include: Blister packs, Bottles, Ampoules, and Vials. The package must include the following essential information: drug name, strength, batch number, manufacturing date, expiry date, and storage conditions.



Figure 06: Tablet packaging and filling machine inside the manufacturing facility.

1.5.9. Storage and Distribution

Pharmaceuticals are stored after manufacturing under strict conditions to maintain their stability and quality until they reach the patient. Storage conditions generally include: appropriate temperature, low humidity, protection from light, and prevention of contamination. This stage is considered important because some chemical substances may degrade when exposed to high temperatures or humidity, leading to a reduction in their efficacy or the formation of harmful degradation products.



Figure 07: Storage warehouse of drugs inside the manufacturing plant or central warehouse.

2. Biologic Drugs Manufacturing Process

The active substance in biologic drugs consists of recombinant proteins produced using microorganisms or living cells such as bacteria, yeasts, plant cells, or animal and human cells, in order to produce proteins, enzymes, antibodies, or other pharmacologically active

molecular products. Their manufacturing process differs significantly from that of chemical drugs (Table 1), as it relies on highly controlled biological conditions and strict monitoring of microbial contamination and the biological activity of the produced substance.

2.1. Gene and Cell Line Selection

➤ Gene of Interest Selection

- The gene encoding the desired protein is identified, taking into account its ability to be expressed in the selected host cell and its efficiency in protein production.
- The gene may be genetically modified if necessary to improve expression levels, protein folding, or post-translational modifications .

➤ Cell Line Selection

The host cells into which the gene is introduced for protein production are selected. These cells act as a “biological factory” that reads the gene and translates it into a protein. Selection is based on:

- o High protein production capacity
- o Genetic stability of the cell bank
- o Ability to perform post-translational modifications
- o Ease of growth in large-scale industrial bioreactors

❖ Examples of Cell Lines Used

1. Mammalian cells:

- o **CHO (Chinese Hamster Ovary Cells):** most widely used globally for recombinant proteins and therapeutic antibodies due to high productivity, excellent quality, and genetic stability.
- o **NS0 (Mouse Myeloma Cells):** used for antibody production; good production but less precise post-translational modifications.
- o **BHK (Baby Hamster Kidney Cells):** used for some proteins and vaccines.

2. Human cells:

- o **HEK293 (Human Embryonic Kidney 293 Cells):** used for proteins requiring precise

human glycosylation or for producing genetically engineered viruses.

o **PER.C6 (Human Retinal Cells):** used for proteins and vaccines requiring precise glycosylation.

3. Insect cells (Sf9, Sf21):

Used with baculovirus systems to produce proteins that do not require complex human-like glycosylation.

4. Plant or fungal cells:

Used for large-scale production of some proteins, but less common in human pharmaceuticals.

5. Bacterial cells (e.g., *E. coli*):

Characterized by rapid growth and low production cost. They are used to produce simple proteins that do not require post-translational modifications, such as certain enzymes and small proteins. However, bacteria do not usually establish complex cell banks such as Master Cell Bank (MCB) or Working Cell Bank (WCB), because they are not capable of performing complex biological modifications. Therefore, their use is mainly for direct or limited batch production. This explains why complex cell banking systems are mainly applied to mammalian, human, and insect cells, while bacteria are considered a cost-effective option for rapid production of simple proteins.

❖ Most Widely Used Global Cell Lines

- **CHO:** for protein and antibody production due to high protein quality and process stability.
- **HEK293:** when precise human-like protein modifications are required.

➤ Gene–Cell Compatibility

- The gene is introduced into different cells using a vector.
- Protein expression level, purity, and functional stability are evaluated.
- Cells producing the best yield and highest-quality recombinant protein are selected.

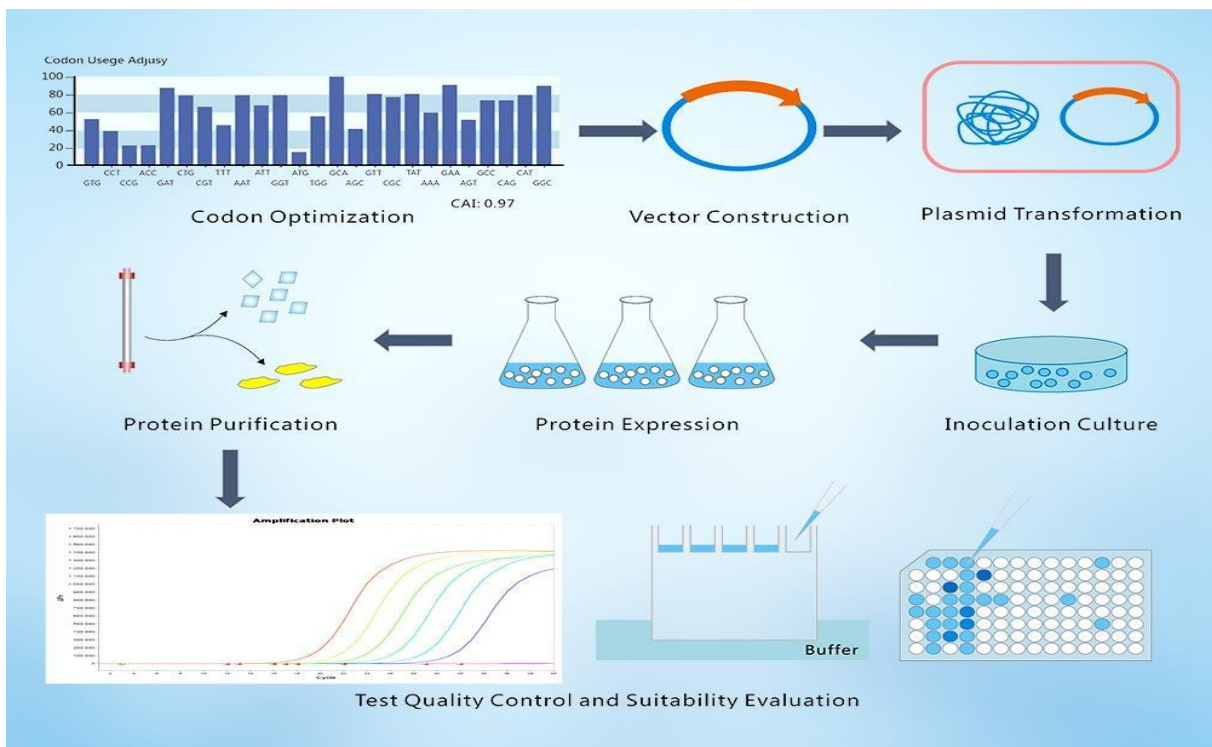
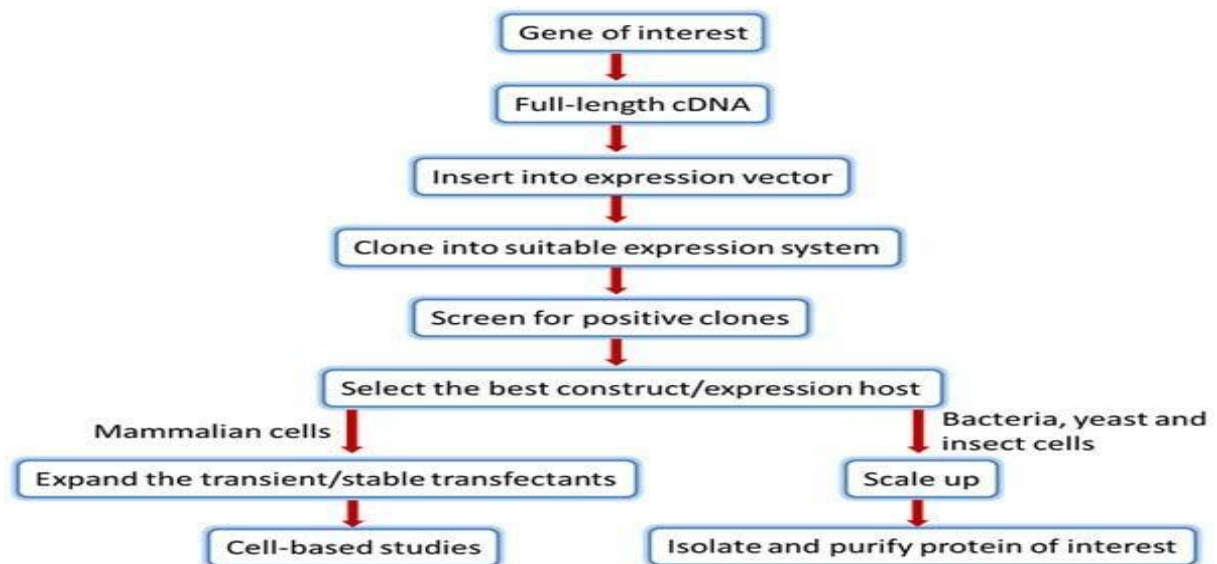


Figure 08: Several types of cells used in recombinant protein production, as well as the process of gene identification encoding the target protein, including the assessment of its expression capacity in the selected cell line.

2.2. Cell Lines and Cell Banks

- A cell line is a population of genetically identical cells that have the ability to continuously grow and divide under laboratory conditions while maintaining their capacity to produce the target protein.

- After gene insertion, a large population of cells is obtained; however, not all cells produce the protein with the same efficiency. Therefore, the process includes the following stages:
 - o Isolation of successfully expressing cells.
 - o Evaluation of protein production level for each cell.
 - o Selection of the highest producing and most stable cells.
 - o Optimization of environmental conditions to increase production.

This process is called **cell selection**, and it is essential to obtain a stable and efficient production line.

- After obtaining a stable cell line, it is preserved in the form of “cell banks” to ensure continuous availability during industrial production.
- The **Master Cell Bank (MCB)** is established once after selecting the ideal cell line and is stored under deep freezing conditions (cryopreservation). It is used to generate all future production batches to ensure product consistency in quality and characteristics.
- The **Working Cell Bank (WCB)** is derived from the Master Cell Bank and is used for industrial production. The Master Cell Bank is only accessed again when a new Working Cell Bank needs to be generated.
- The Master Cell Bank is stored to allow the generation of Working Cell Banks for future industrial production when required.
- This hierarchy ensures that all production is based on genetically stable, pure, and reliable cells that produce identical recombinant protein batches with consistent efficiency and stability.
- Cell banks are stored at extremely low temperatures (usually -196°C using liquid nitrogen), which stops all cellular activity and preserves their characteristics without alteration. Continuous verification of cell line stability and strict monitoring of storage conditions are required to prevent genetic drift over time or loss of cellular productivity.

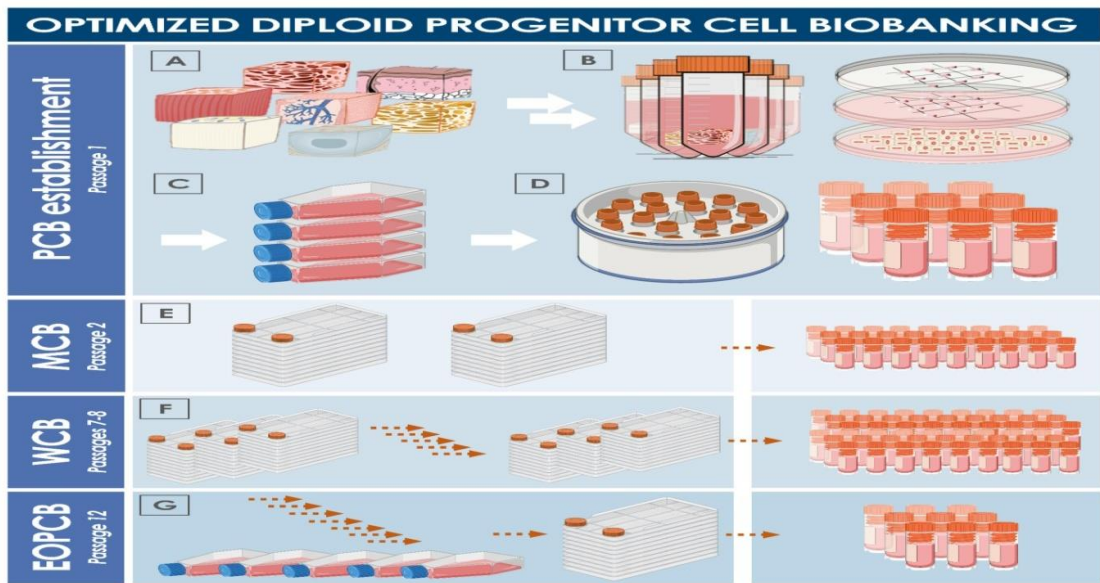
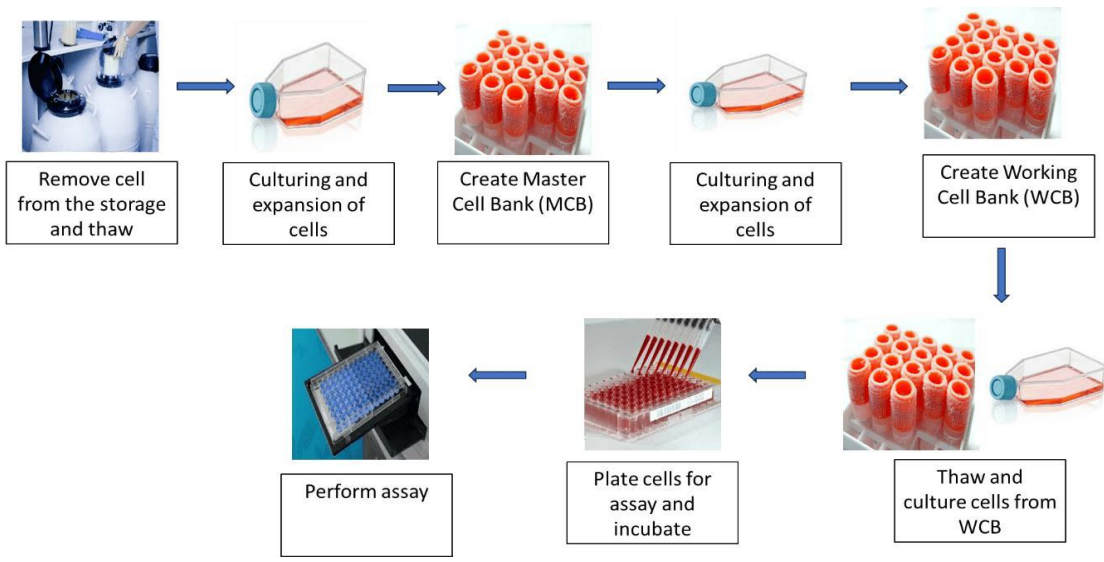
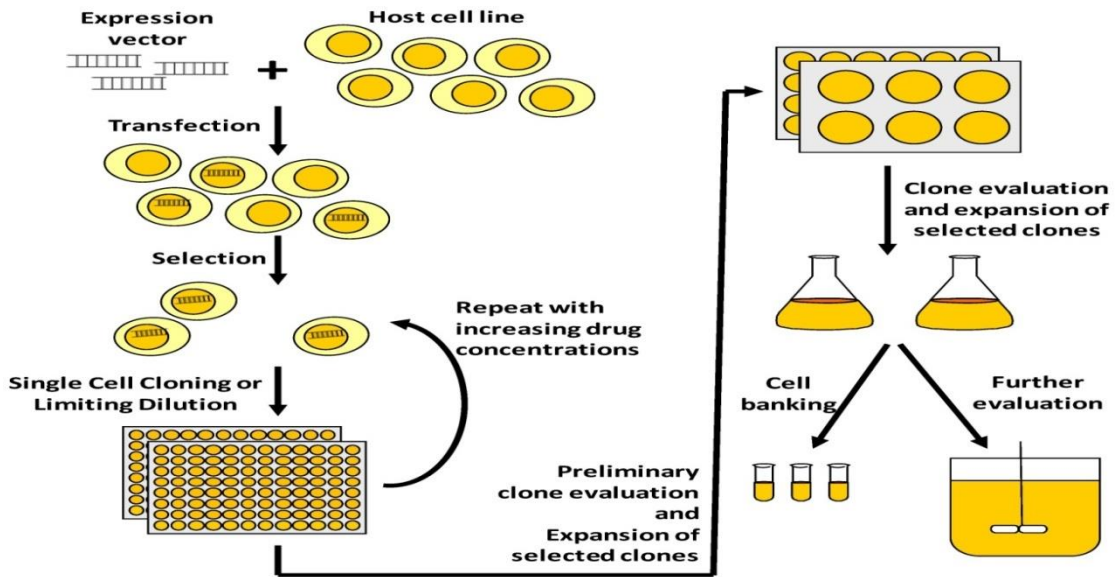


Figure 09: Process of generating genetically modified cell lines after insertion of the gene of interest.

2.3. Bioreactor Cell Culture and Recombinant Protein Expression (Upstream Processing)

- WCB cells are cultured in bioreactors under optimal conditions: temperature, pH, oxygen level, and nutrient concentration.
- Inside the bioreactor, cells begin to grow and proliferate in a controlled manner. Upon reaching the appropriate density, they activate the inserted gene and produce the recombinant protein. This protein is either secreted into the intracellular compartment or into the surrounding medium depending on the expression system used. The process is continuously monitored to ensure cell stability and product quality.
- Cell growth rate, protein production, and medium purity are monitored to ensure the quality of the final product.

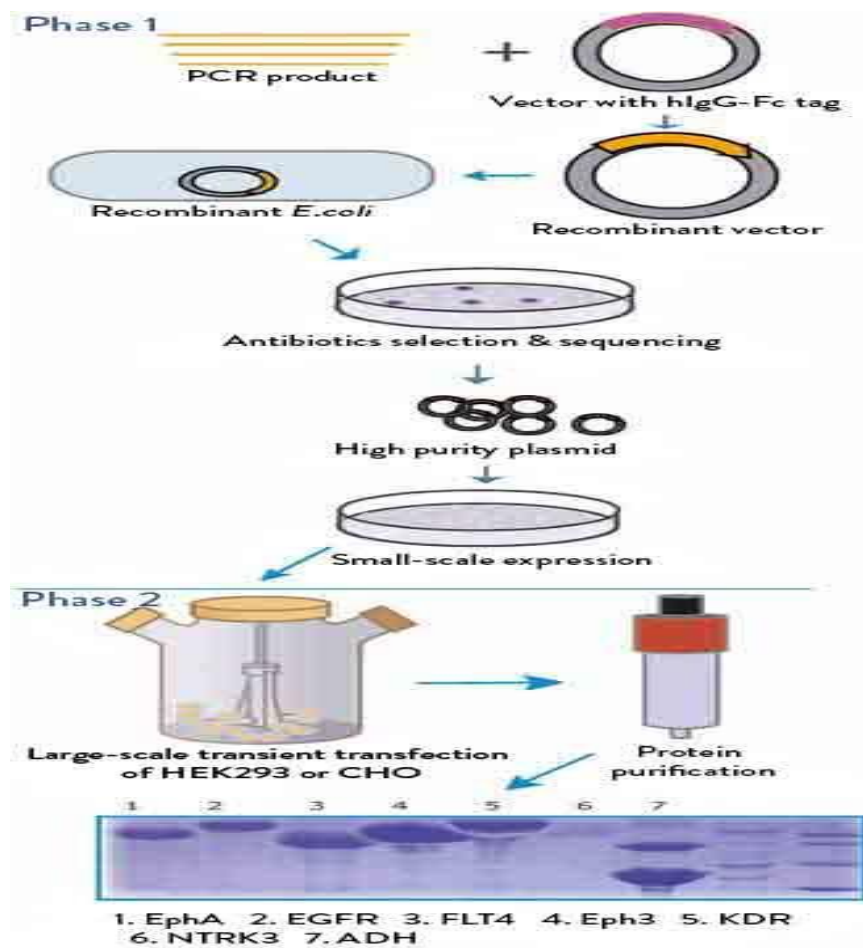


Figure 10: The inoculation of Working Cell Bank (WCB) cells into bioreactors under optimal conditions.

2.4. Separation and Purification of Recombinant Protein (Downstream Processing)

• After production, the recombinant protein is separated from cells, impurities, and culture media in order to obtain a pure, stable, and biologically active product:

- o Filtration to remove dead cells and large particles.
- o Chromatography to isolate the pure protein from other proteins and trace impurities.
- o Concentration to adjust protein levels for the final formulation stage.

Affinity Chromatography: another view

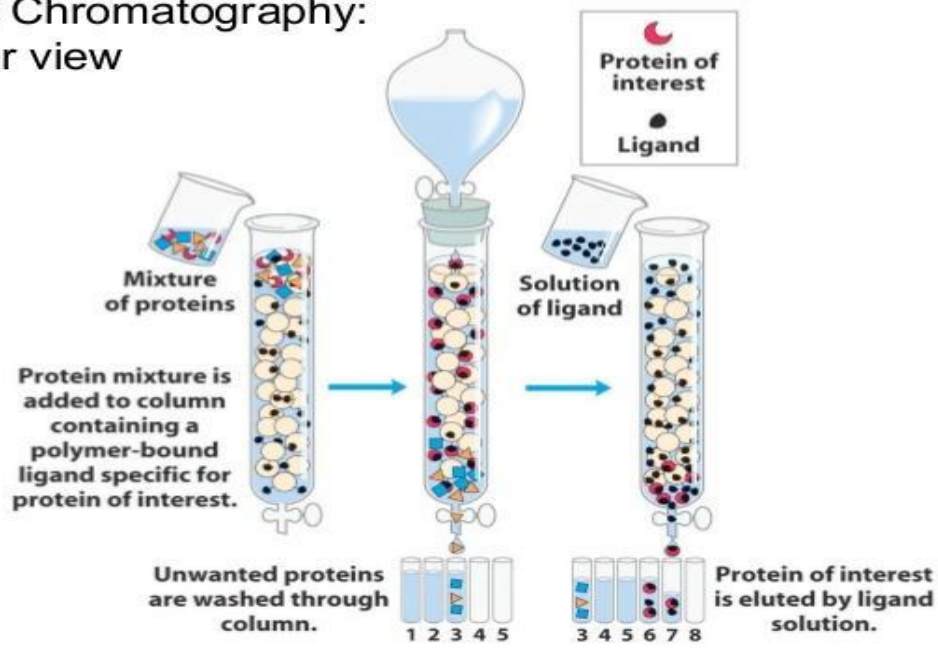


Figure 11: The use of chromatography to isolate the pure protein from other proteins and trace impurities.

2.5. Quality Control and Bioassays of Recombinant Protein

Quality control and bioassays (tests used to measure the biological activity or potency of a pharmaceutical substance, particularly recombinant proteins) represent a critical stage in biopharmaceutical manufacturing. This stage aims to ensure that the produced recombinant protein meets the required specifications in terms of purity, identity, potency, and safety before its use in the final medicinal product. During this stage, a series of laboratory tests are performed, including purity assessment to confirm the absence of impurities, identity testing to verify that the protein corresponds to the intended molecule, and bioactivity assays to

measure the therapeutic functionality of the protein. Safety tests are also conducted to detect any toxins, microbial contamination, or viral presence, in addition to stability studies that evaluate protein stability during storage and transport. This stage is critical as it ensures the quality, safety, and efficacy of the final drug product prior to its release for use.

2.6. Final Drug Formulation

The final formulation stage is a key step in biopharmaceutical manufacturing, where the purified recombinant protein is converted into a finished dosage form suitable for therapeutic use without altering its activity. In this stage, the protein is mixed with excipients, which are inactive substances that are essential to maintain drug stability and protect the protein from degradation or loss of activity during storage and transport. These excipients include protein stabilizers, pH-adjusting agents, preservatives that prevent microbial growth, and compounds that prevent protein aggregation or provide protection such as sugars. Together, these components ensure that the protein remains in its active form and retains its quality until the time of administration, making the drug safe and effective for patient use.

2.7. Filling and Packaging

The filling and packaging stage is the final step in biopharmaceutical manufacturing, where the finished drug is prepared for distribution and use while maintaining its quality and stability. In this stage, the recombinant protein is filled into suitable pharmaceutical containers such as glass vials, pre-filled syringes, or ampoules, using inert materials that do not interact with the active substance. Secondary packaging is also applied to protect the product during transport and storage and to facilitate distribution. The process is carried out under fully aseptic conditions, with protection against light, temperature variations, and contamination. Labeling is applied to the container, including essential information such as drug name, concentration, manufacturing and expiry dates, batch number, storage conditions, and instructions for use. A package leaflet is also included, providing information on correct usage, indications, dosage, warnings, side effects, and proper storage conditions to ensure safe and effective use. This stage is essential to ensure that the drug reaches the patient with the same quality and efficacy as produced, while maintaining its safety and stability until administration.

2.8. Storage and Distribution

The storage and distribution stage is one of the final steps in biopharmaceutical manufacturing, ensuring the maintenance of drug quality and stability after packaging until it reaches the patient. Due to the sensitivity of recombinant proteins, these products are stored under strict and controlled conditions, typically at low temperatures (usually 2–8°C or deep-freezing depending on the product), while avoiding exposure to light, heat, and mechanical stress, and maintaining a sterile environment. A cold chain system is implemented to ensure that the product remains within the required temperature range throughout transport and storage using cold rooms, refrigerated trucks, and insulated containers equipped with monitoring systems. The drugs are then distributed to hospitals, pharmacies, and healthcare centers under the same storage conditions to ensure product integrity. This stage is essential to preserve the biological activity of the protein, prevent degradation or loss of function, and ensure that the drug reaches the patient with full quality and therapeutic efficacy.

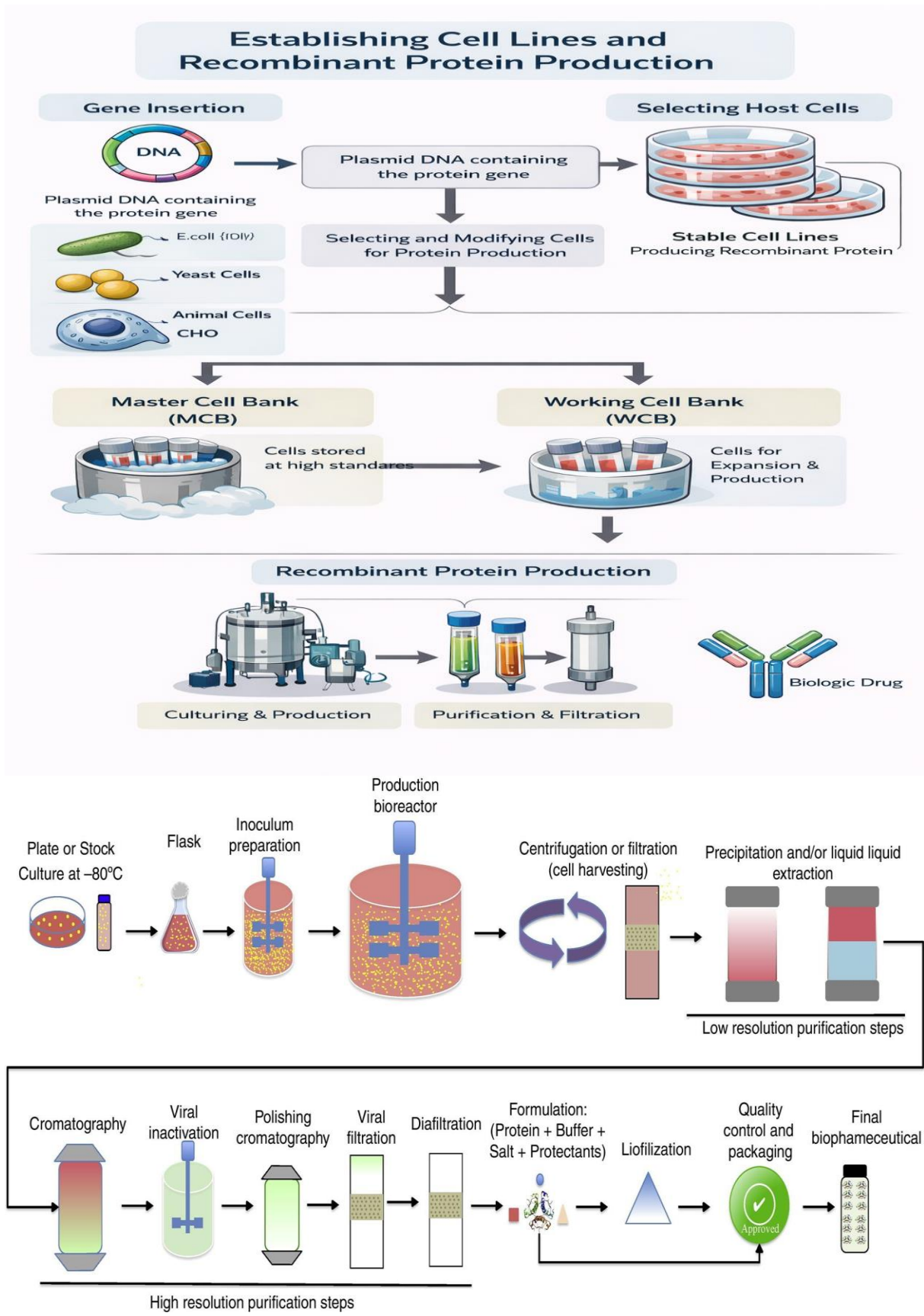


Figure 12: The preparation of cells or microorganisms used for the production of biological substances.

3. Key Differences Between Biologic Drugs and Chemical Drugs

Although biologic drugs and chemical drugs share the common goal of treating diseases and improving patient health, they differ fundamentally in molecular structure, manufacturing processes, stability, as well as pharmacological and clinical properties, as shown in Table 01, which presents the specific characteristics of each type, highlighting their advantages and limitations in the context of modern drug development.

Table 01: Comprehensive Comparison Between Biological Drugs and Chemical Drugs

Property	Biological Drugs	Chemical Drugs
Molecular size	Large and complex	Small and simple
Structure	Proteins, antibodies, enzymes	Pure chemical compounds
Production source	Living cells or microorganisms (bacteria, yeasts, animal/human cells)	Industrial chemical reactions
Manufacturing methods	Bioreactors, cell banks, strict monitoring	Industrial laboratories, standardized steps
Stability	Sensitive to heat, light, and pH	Generally stable at room temperature
Route of administration	Injection or intravenous infusion	Oral, topical, or injection depending on need
Quality control	Protein purity, post-translational modifications, microbial contamination	Purity and concentration of the compound
Post-translational modifications	Essential for protein biological activity	Not required
Immunogenicity	May occasionally trigger immune response	Usually does not induce immune response
Personalized medicine	Can be designed for targeted and precise therapy	Broad-spectrum treatment, less specific
Cost and time	Very high cost, longer production time	Lower cost, faster production
Regulation and control	Highly strict, monitored by agencies such as FDA and EMA	Relatively simpler
Environmental sensitivity	Highly sensitive to temperature, humidity, and light; requires cold chain	More stable, usually no strict refrigeration required

Property	Biological Drugs	Chemical Drugs
Shelf life	Relatively short	Longer
Copying ability	Biosimilars can be produced but are not identical	Generic drugs are identical copies
Drug interactions	Limited and specific due to targeted action	More prone to interactions with drugs or food
Use in diseases	Mainly chronic or rare diseases	Common diseases or conventional therapy
Microbial contamination control	Strict monitoring at all stages; minor contamination can compromise the batch	Relatively low risk
Long-term patient effect	May induce anti-drug antibody formation	Long-term effects related to toxicity or accumulation
Production batch size	Requires long time and large bioreactor capacity	Large batches produced quickly
Dose precision control	Requires strict monitoring due to protein sensitivity	More stable dosing
Formulation complexity	Special buffers, sometimes immunosuppressive excipients	Relatively simple formulations
Future innovation	Cell therapies, gene therapy, monoclonal antibodies	Improved bioavailability, reduced side effects, new chemical entities
Adaptability to new diseases	Protein/antibody modification possible but complex	New molecule development takes longer

4. Uses of Biological Drugs Compared to Chemical Drugs

Chemical drugs and biological drugs are considered fundamental pillars of modern medicine for the treatment, prevention, and control of diseases. The choice between them depends on the nature of the disease and its underlying pathophysiological mechanism.

Chemical drugs (Small Molecule Drugs) are small chemical compounds designed to modulate a specific biological function or to treat a defined disease. They are characterized by rapid

interaction with drug targets and relatively stable physicochemical properties, with the possibility of precise dose control. They also offer large-scale and low-cost production and multiple routes of administration (oral, injectable, topical, inhalation), in addition to a well-established clinical safety and efficacy record. These drugs mainly act on receptors or enzymes by inhibiting or activating biological pathways; therefore, they are widely used in the treatment of infectious diseases, pain, inflammation, and cardiovascular disorders. However, their limited selectivity may lead to adverse effects on non-target organs, as well as the possible development of drug resistance. In contrast, biological drugs (Biopharmaceuticals) are large molecules such as recombinant proteins, antibodies, and enzymes produced using living cells. Their activity depends on biological stability, post-translational modifications, and immunological compatibility. They are characterized by high target specificity and strong efficacy, particularly in cases resistant to chemical drugs. They are also capable of producing complex molecules similar to endogenous biological structures and may reduce side effects in certain cases. Biological drugs are mainly used in the treatment of cancer (especially immunotherapy), autoimmune diseases, and hormonal disorders such as recombinant insulin therapy, in addition to vaccines and genetic diseases through enzyme or protein replacement. However, they require special storage conditions (cold chain) and are associated with high production costs due to the complexity of manufacturing processes.

Shared Therapeutic Applications

Both chemical and biological drugs are essential therapeutic tools used in:

- Treatment of various diseases, whether acute or chronic.
- Cancer therapy, despite differences in mechanism (chemical cytotoxic vs immune/targeted therapy).
- Inflammatory and immune diseases by reducing inflammation or modulating immune response.
- Infectious diseases such as viral and bacterial infections (via different mechanisms).
- Hormonal disorders (e.g., insulin and growth hormone disorders).
- Neurological and psychiatric disorders (e.g., depression, epilepsy, psychosis).
- Support or improvement of physiological functions through modulation of internal biological pathways.

Both types aim to treat or control disease, but through different mechanisms as shown in Table 02.

Table 02: Differences in the Uses of Biological Drugs and Chemical Drugs

Field	Chemical Drugs	Biological Drugs
Nature of use	General disease and symptom treatment	Targeted and precise treatment of molecular causes
Mechanism of action	Broad pathway modulation (enzymes/receptors)	Highly specific targeting of molecules or cells
Chronic diseases	Symptom control (diabetes, hypertension)	Used in complex or resistant cases
Cancer	Non-selective killing of rapidly dividing cells	Targeted cancer cells or immune activation
Immune diseases	General immunosuppression or anti-inflammatory action	Selective inhibition of molecules such as TNF- α and IL-6
Infectious diseases	Antibiotics/antivirals	Specific antibodies to neutralize pathogens
Hormonal disorders	General hormone replacement or stimulation	Recombinant proteins such as insulin and growth factors
Degree of precision	Lower precision (broad effect)	High precision (targeted therapy)
Side effects	More likely due to non-selective action	Relatively fewer due to specificity
Onset of action	Usually rapid, short to medium duration	Slower onset, long-lasting and targeted effect

5. Conclusion

The manufacturing of drugs, whether chemical or biological, represents one of the fundamental pillars of the modern pharmaceutical industry, aiming to provide effective and safe therapeutic agents for disease prevention, treatment, and improvement of patient quality of life. Chemical drugs are based on chemical synthesis processes starting from simple raw materials through controlled chemical reactions, enabling the production of small molecules with defined and relatively stable structures that are easy to manufacture on a large scale. In contrast, biological drugs rely on living systems such as bacterial, animal, or yeast cells to produce complex proteins such as antibodies, hormones, and enzymes, making their manufacturing process more complex and requiring highly controlled biological conditions and strict monitoring.

Despite differences in manufacturing approaches, both types share the same goal of developing effective therapies targeting a wide range of diseases, including chronic diseases, cancers, immune disorders, inflammatory conditions, and infectious diseases. Chemical drugs are characterized by ease of production and wide therapeutic use, while biological drugs are distinguished by high targeting precision and effectiveness in treating complex diseases that are difficult to manage using conventional approaches.

Ultimately, the integration of chemical and biological drugs reflects the advancement of pharmaceutical sciences, where combining both approaches has become essential to expand therapeutic options and achieve better health outcomes, while ongoing technological progress continues to improve drug safety and efficacy in the future.

Chapter II

II. Quality Control in Biopharmaceutical Manufacturing

Quality control in biopharmaceutical manufacturing is an essential component of the production system. Its role is not limited to final product testing but extends across all manufacturing stages, starting from gene and cell line selection, through production processes in bioreactors, and continuing to purification and final formulation steps. This type of control responds to the sensitive and complex nature of biopharmaceutical products, such as recombinant proteins and monoclonal antibodies, where any minor variation in production conditions may lead to changes in the three-dimensional structure or biological function of the active substance. Therefore, biopharmaceutical manufacturing relies on a strict and integrated control system aimed at ensuring safety, efficacy, and stability, in full compliance with international pharmaceutical quality standards. This control system is based on a combination of advanced analytical techniques, including physicochemical, microbiological, immunological detection methods, and toxicity testing, to ensure that the final product fully meets the required therapeutic specifications. Accordingly, quality control techniques form a critical link between the production stage and the clinical application stage, ensuring that the drug is transferred from manufacturing to the patient with the highest level of safety and quality.

1. Physicochemical Control Techniques

Physicochemical control techniques are fundamental tools in monitoring the quality of biopharmaceutical products. They are used to evaluate the physical and chemical properties of produced proteins or biological molecules and to ensure their compliance with predefined specifications before, during, and after manufacturing. These techniques are particularly important in the biological field because protein-based products are highly sensitive to environmental conditions such as temperature, pH, and pressure, which may alter their three-dimensional structure and consequently reduce their therapeutic activity. The main objectives of these techniques are to ensure product identity, purity, and stability by assessing Critical Quality Attributes (CQA), also referred to as critical control parameters, such as tertiary structure, biological activity, and impurity levels. Monitoring is not limited to the final product; it also includes in-process control within bioreactors, where physical and chemical changes are tracked in real time to ensure that production remains within predefined limits.

1.1 Physical Control Techniques

Physical control techniques focus on studying the structural and behavioral properties of biological materials without altering their chemical composition. They are essential for evaluating protein stability and conformational integrity.

The main techniques include:

- **Structural spectroscopy analysis** (e.g., FTIR and Circular Dichroism) to study the secondary and tertiary structures of proteins.
- **Electrophoresis (SDS-PAGE)** to separate proteins according to molecular weight and verify structural integrity.
- **Particle size analysis** to determine the homogeneity of protein molecules.
- **Viscosity measurement**, particularly important for protein solutions and injectable formulations, as increased viscosity may hinder administration.
- **Physical stability tests** to detect aggregation or conformational changes.



Figure 13: The method of introducing the sample into a viscometer to determine its flowability.

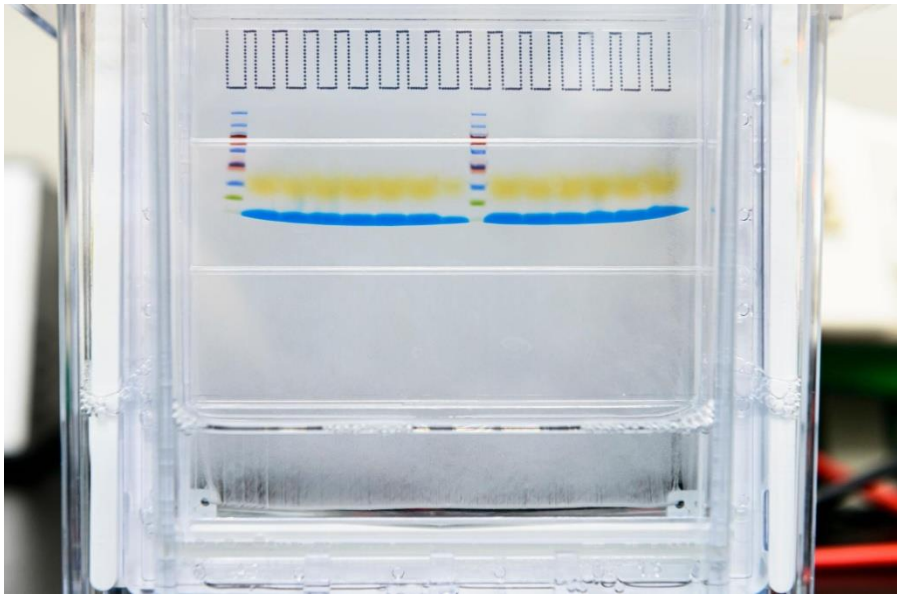


Figure 14: Structural analysis techniques such as SDS-PAGE and spectroscopic analysis.

1.2 Chemical Control Techniques

Chemical control techniques aim to analyze the chemical composition of proteins and impurities and to ensure correct identity, purity, and molecular structure.

The main techniques include:

- **High Performance Liquid Chromatography (HPLC):** used to separate compounds and determine purity and impurity levels.
- **UV-Visible Spectroscopy (UV-Vis):** used for concentration determination and chemical identity confirmation.
- **PH measurement:** used to ensure chemical stability of the protein environment.
- **Chemical bond analysis:** used to detect any changes in molecular structure.
- **Oxidation and degradation studies:** used to evaluate the stability of the active substance.

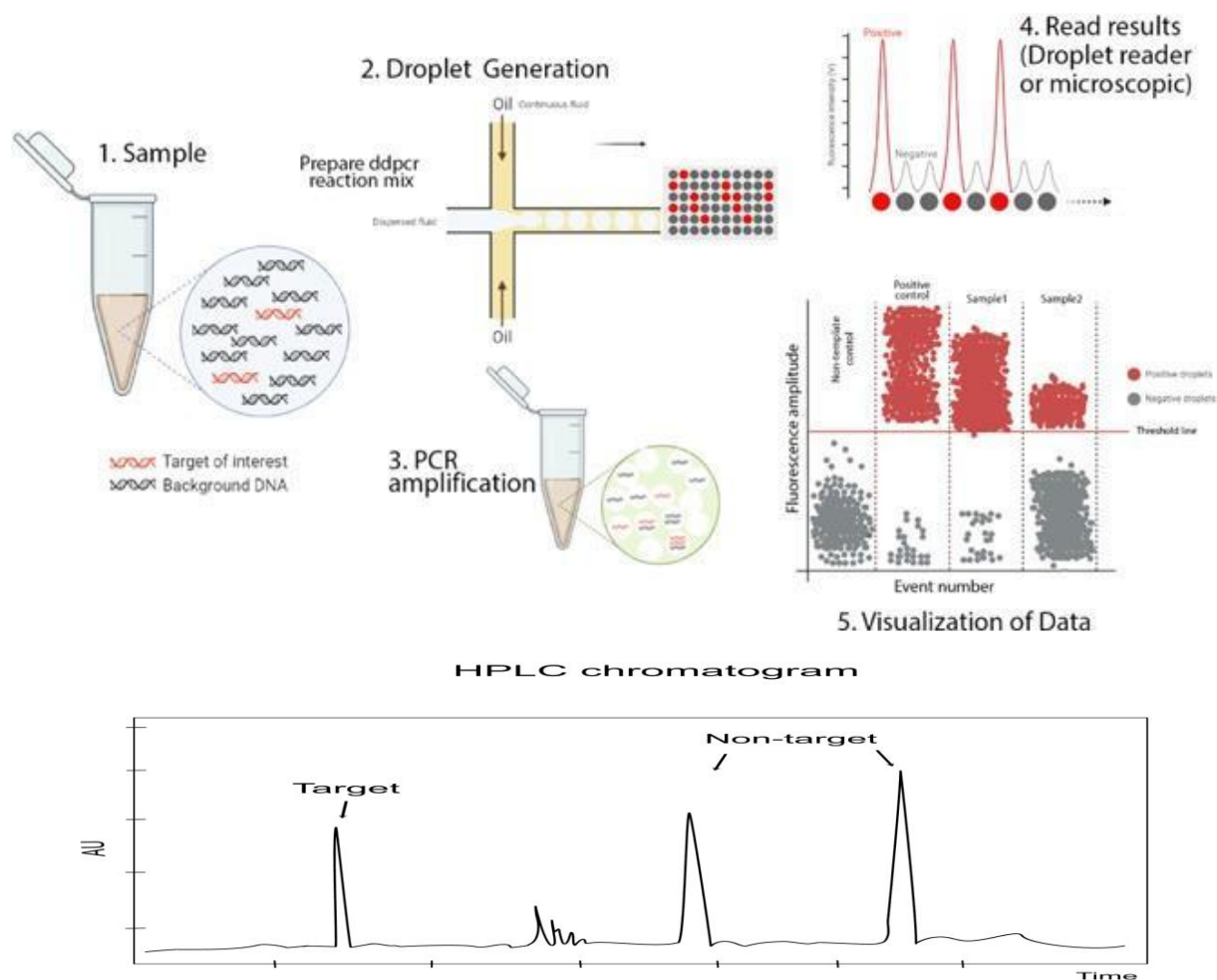


Figure 15: HPLC chromatogram used for chemical purity analysis and high-precision separation of components in biopharmaceutical products.

Although physicochemical techniques differ in their nature, they are used in an integrated manner, as relying on a single approach is not sufficient to ensure the quality of biopharmaceutical products. Instead, both approaches are combined to provide a comprehensive assessment of structure and function. Despite the importance of physicochemical analyses, they are not sufficient on their own to guarantee the safety of biopharmaceutical products. Therefore, they must be integrated with microbiological control techniques, immunological detection methods, and toxicity assays to obtain a complete and accurate evaluation of quality and safety. This stage is also essential for detecting any deviations that may occur during manufacturing, allowing early correction before reaching the final production stages.

2. Microbiological Control Techniques

2.1 Concept of Microbiological Control

Microbiological control refers to a set of laboratory and technical procedures used to detect the presence of microorganisms or limit their growth at different stages of drug manufacturing, particularly in biopharmaceutical products. This control includes monitoring bacteria, fungi, yeasts, and in some cases viruses and mycoplasma, whether in raw materials, during production, or in the final product.

2.2 Importance of Microbiological Control in Biopharmaceutical Manufacturing

Microbiological control is a fundamental element in ensuring the quality and safety of biopharmaceutical products, as these products are often produced using living cells or sensitive biological materials, making them more susceptible to contamination compared to conventional chemical drugs.

Its importance includes:

- Prevention of batch failure due to contamination.
 - Protection of patients from serious complications such as infections, fever, or septic shock.
 - Maintenance of stability and efficacy of therapeutic proteins and vaccines. (Stability of Biologics).
 - Compliance with regulatory requirements and international standards such as GMP (Good Manufacturing Practices), USP (United States Pharmacopeia), and ICH (International Council for Harmonisation).
- Microbiological control is essential in biopharmaceutical manufacturing, as its role is not limited to detecting contamination only, but also extends to prevention, control, and regulatory compliance, making it a critical factor in patient safety and final product quality.

2.3 Sources of Microbial Contamination

Sources of microbial contamination are among the most important factors affecting the quality of biopharmaceutical products, as microorganisms may enter the product at any stage of manufacturing, from raw materials to final packaging.

The most important sources can be summarized as follows:

- **Raw materials:** may contain bacteria, fungi, or microbial impurities if they are not properly processed.
- **Environment:** includes air, water, surfaces, and ventilation systems inside production rooms.
- **Personnel:** are considered a primary source of contamination through skin, clothing, or poor compliance with sterilization procedures.
- **Equipment and manufacturing process:** any failure in sterilization or non-sterile transfer between stages may lead to contamination.

Therefore, the pharmaceutical industry relies on a strict regulatory system aimed at monitoring every possible point of contaminant entry.

2.4. Monitoring of Potential Sources of Microbial Contamination

2.4.1. Raw Material Monitoring

Raw material monitoring is considered a fundamental step in the manufacturing of biologic drugs, as it aims to ensure the quality and safety of all raw materials used before entering the production process, because any contamination in them can be directly transferred to subsequent stages and affect the quality and efficacy of the final drug. Raw materials in this context include water in its two types (Purified Water and Water for Injection), and culture media intended for cell culture which contain components such as amino acids, vitamins, salts, and glucose, in addition to animal serum such as fetal bovine serum which provides growth factors and proteins necessary to support cell proliferation, and

also includes excipients such as stabilizers, pH regulators, preservatives, and emulsifiers used to improve the stability and quality of the pharmaceutical product, in addition to primary biological materials such as living cells, microorganisms, plasmids, or viral vectors which constitute the basis through which the active substance is produced. Gases such as carbon dioxide, oxygen, and nitrogen may also be used to control growth conditions inside bioreactors. These materials may carry bacteria, fungi, viruses, or even endotoxins resulting from bacterial lysis. To ensure the safety of these materials, they are subjected to precise tests including Bioburden testing to estimate the number of microorganisms, Endotoxin Test (LAL) to detect bacterial endotoxins, and qualitative tests to ensure the absence of pathogenic microorganisms, in addition to virus detection techniques such as PCR in biologically derived materials. The importance of this monitoring lies in preventing the introduction of any contamination source from the beginning and ensuring the continuity of the production of safe, effective biologic drugs compliant with international quality standards.

2.4.2. Monitoring of Manufacturing Environment

Environmental monitoring is considered one of the most important elements of quality assurance in the manufacturing of biologic drugs, as it aims to control all sources of contamination present within production areas such as air, surfaces, and water, because these elements can transfer microorganisms to the product during manufacturing. This monitoring is mainly based on cleanroom systems designed according to ISO standards to ensure the reduction of particles and contaminants to the lowest possible level, with strict application of ventilation, sterilization, and airflow control procedures. Air is monitored using particle counters that measure the number and size of non-viable particles, and air samplers that detect viable microorganisms, as these techniques help ensure that the HVAC system operates efficiently to prevent the entry of contaminants. Surfaces are also examined using swabs to collect samples from surfaces or using contact plates to detect any microbial contamination. Water used in manufacturing such as Purified Water and Water for Injection is periodically monitored through bioburden tests, endotoxin tests, and chemical properties (Conductivity & TOC), in addition to monitoring the possible formation of biofilms inside pipes and tanks. The importance of this monitoring lies in ensuring a sterile or low-contamination production environment, thereby reducing the risk of spoilage of biologic products and maintaining their efficacy and safety, while strictly complying with GMP and ISO standards to ensure

production quality. 2.4.3. Personnel and Equipment Monitoring Personnel and equipment monitoring are considered vital elements in the manufacturing of biologic drugs, because humans and equipment represent among the most important sources of contamination within the production environment, even when high-quality cleanrooms are available. Therefore, strict systems are applied to control the movement of personnel within manufacturing areas, with the obligation to wear special sterile garments that cover the entire body to reduce the transfer of microorganisms from skin, hair, or clothing. The microbial burden of personnel is also periodically assessed using swabs taken from hands and specific areas of clothing, or using contact plates that detect microorganisms transferred during work. In addition, personnel are trained on cleanroom entry and exit rules and on the application of aseptic working practices to avoid any accidental contamination. As for equipment, it is considered a potential source of contamination if not properly maintained and sterilized; therefore, it is subjected to strict programs including Cleaning Validation and Sterilization Validation before and after use, with periodic monitoring for any microbial residues or biofilm formation inside internal surfaces of equipment and production lines. Equipment integrity is also verified before operation to ensure that no contaminants are transferred into the product during the manufacturing process. The importance of this monitoring lies in ensuring that no human or technical contamination is introduced into the production process, thereby enhancing the quality and safety of biologic drugs and maintaining compliance with international standards such as GMP.

2.4.2. Manufacturing Environment Monitoring

Environmental monitoring is considered one of the most important elements of quality assurance in the manufacturing of biologic drugs, as it aims to control all sources of contamination present within production areas such as air, surfaces, and water, because these elements can transfer microorganisms to the product during manufacturing. This monitoring is mainly based on cleanroom systems designed according to ISO standards to ensure the reduction of particles and contaminants to the lowest possible level, with strict application of ventilation, sterilization, and airflow control procedures. Air is monitored using particle counters that measure the number and size of non-viable particles, and air samplers that detect viable microorganisms, as these techniques help ensure that the HVAC system operates efficiently to prevent the entry of contaminants. Surfaces are examined using swabs to collect

samples from surfaces or using contact plates to detect any microbial contamination. Water used in manufacturing such as Purified Water and Water for Injection is periodically monitored through bioburden tests and endotoxin tests , as well as chemical properties (Conductivity & TOC), in addition to monitoring the possible formation of biofilms inside pipes and tanks. The importance of this monitoring lies in ensuring a sterile or low-contamination production environment, thereby reducing the risk of spoilage of biologic products and maintaining their efficacy and safety, while strictly complying with GMP and ISO standards to ensure production quality.

2.4.3. Personnel and Equipment Monitoring

Personnel and equipment monitoring are considered vital elements in the manufacturing of biologic drugs, because humans and equipment represent among the most important sources of contamination within the production environment, even when high-quality cleanrooms are available. Therefore, strict systems are applied to control the movement of personnel within manufacturing areas, with the obligation to wear special sterile garments that cover the entire body to reduce the transfer of microorganisms from skin, hair, or clothing. The microbial burden of personnel is also periodically assessed using swabs taken from hands and specific areas of clothing, or using contact plates that detect microorganisms transferred during work. In addition, personnel are trained on cleanroom entry and exit rules and on the application of aseptic working practices to avoid any accidental contamination. As for equipment, it is considered a potential source of contamination if not properly maintained and sterilized; therefore, it is subjected to strict programs including Cleaning Validation and Sterilization Validation before and after use, with periodic monitoring for any microbial residues or biofilm formation inside internal surfaces of equipment and production lines. Equipment integrity is also verified before operation to ensure that no contaminants are transferred into the product during the manufacturing process. The importance of this monitoring lies in ensuring that no human or technical contamination is introduced into the production process, thereby enhancing the quality and safety of biologic drugs and maintaining compliance with international standards such as GMP.

2 . 4. 4. In-Process Monitoring and Verification of Bioburden Control

In-process monitoring is considered a fundamental stage within biological monitoring techniques, and it aims to ensure control of product quality during all stages of manufacturing and not only at the beginning and/or at the end, where this is achieved through continuous monitoring of operational conditions within bioprocess production lines such as temperature, pH, dissolved oxygen, and cell density, particularly inside bioreactors which are considered the main environment for the production of the active substance, and measurement of bioburden is also considered part of in-process monitoring and is performed at intermediate stages to ensure that no contamination occurs during transfer between different manufacturing steps, with the application of rapid microbiological methods for early detection of any abnormal change in microbiological quality, and Critical Control Points are adopted to monitor any deviation that may affect the quality of the final product such as partial sterilization failure or contamination of the medium during transfer or preparation, with the use of advanced analytical techniques such as PCR or rapid measurement systems to detect contaminants in a short time instead of traditional methods, and the importance of this stage lies in enabling immediate intervention to correct any defect before the product reaches the final stages, thereby ensuring compliance of the product with global quality and safety standards such as GMP and GLP.

In this context, bioburden control is considered a comprehensive process that combines in-process monitoring and verification of the effectiveness of the system as a whole, where verification aims to ensure that the applied procedures are capable of reducing and preventing microbial contamination across all production stages, through evaluation of the effectiveness of cleaning and disinfection processes, filtration systems , clean rooms efficiency, pharmaceutical water systems quality , and raw materials, with reliance on the results of periodic bioburden measurements, environmental monitoring tests, and surface and equipment testing to confirm the effectiveness of the control system, and potential sources of contamination such as personnel, air, raw materials, and equipment are analyzed to determine mitigation mechanisms, and this integration between bioburden measurement during production and verification of the control system is considered a foundation for ensuring the Sterility Assurance Level (SAL), making this stage a pivotal element in building an integrated microbiological quality system that ensures the safety and efficacy of biological drugs.

2. 4. 5. Supportive Safety Monitoring

Supportive safety tests are considered a complementary and essential stage in biological monitoring techniques, as quality control is not limited only to materials, the process, or the final product, but extends to include verification of the efficiency of the manufacturing system itself and its ability to prevent contamination. The most important of these tests include:

2. 4. 5. 1. Aseptic Filling Simulation Monitoring

The aseptic filling simulation test (Media Fill Test) is used to evaluate the effectiveness of the aseptic process and personnel during the final filling stage, where the actual product is replaced with a sterile nutrient medium such as Tryptic Soy Broth (TSB), and then all filling steps are simulated under the same actual production conditions in terms of environment, equipment, and personnel. After that, the containers are usually incubated for 14 days at appropriate temperatures, and it is ensured that there is no microbial growth; the absence of turbidity indicates process success, while its appearance indicates contamination and failure of the aseptic system. This test specifically focuses on the aseptic filling stage (Filling & Finishing) because it is considered one of the most contamination-prone manufacturing stages, since it is often carried out in partially open systems with direct human intervention and exposure to air, unlike previous stages that are performed in closed systems. Therefore, this test is considered an accurate simulation of this critical stage, which represents the last point at which contamination can enter the product before reaching the patient. From a practical perspective, the Media Fill Test is not performed before or after actual filling, but is executed as an independent process that simulates the filling stage itself, where the entire filling line is operated without using the actual product. This test is used as part of aseptic process validation, as it is applied before the start of commercial production, and is repeated periodically throughout the manufacturing lifecycle, as well as when changes are introduced to equipment or procedures, in order to ensure the continued efficiency of the aseptic system and its ability to prevent contamination.

2. 4. 5. 2. Preservative Effectiveness Monitoring Preservative Effectiveness Test – PET

The preservative effectiveness test is considered an important test, especially in multi-dose products, where known amounts of microorganisms are added to the product, and then their

ability to survive or disappear over time is measured (such as day 0, 7, 14, 28). If the number of microorganisms decreases significantly, this indicates the effectiveness of the preservative in preventing microbial growth during storage and use. The importance of this test lies in the fact that it does not only measure contamination, but also evaluates the efficiency of the manufacturing system and the applied procedures, and reveals any defect in sterilization, training, or the working environment before the product is released to the market. Therefore, it is considered an essential part of overall quality assurance in the manufacturing of biological drugs and is applied according to strict standards such as GMP and USP.

2. 4. 6. Final Product Monitoring and Testing

Final product testing is considered the critical and final stage in the series of monitoring and quality assurance of biological drugs, ensuring product safety before being released to the market for use, where it is used to confirm that the product, after completion of all manufacturing stages, is free from any microbial, viral, or endotoxin contamination that may affect patient safety. This stage is also used as a final decision point based on which batch release is accepted, rejected, or reprocessed according to international regulatory standards such as USP, GMP, and ICH. This stage includes a set of essential tests, most importantly the Sterility Test, which aims to detect the absence of any viable microorganisms using membrane filtration or direct inoculation depending on the nature of the product, in addition to the Endotoxin Test (LAL) for detecting endotoxins produced by Gram-negative bacteria even in the absence of viable cells. Microbial load tests are also performed in non-sterile products (TAMC and TYMC) to determine the total count of bacteria and fungi within acceptable limits, along with specified microorganism tests such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, which must be completely absent. This stage also includes specific tests for biological products such as mycoplasma and viral detection using PCR or ELISA techniques. The importance of this monitoring lies in the fact that it represents the final guarantee of product quality, as any defect in previous stages becomes directly evident at this stage, which may lead to rejection of the entire batch, making it a fundamental element to ensure compliance with safety, efficacy, and safe use requirements. In biological drugs, these standards are more stringent compared to chemical drugs due to their reliance on living systems and their high sensitivity to even minimal contamination.

2. 5. Microbiological Tests Used in the Monitoring of Biological Drug Manufacturing

In the manufacturing of biological drugs, especially those intended for parenteral administration, microbiological safety is considered a non-negotiable requirement, as these products must be completely sterile and free from any viable microorganisms upon reaching the final product, due to the fact that they are administered directly into the body or bloodstream. Therefore, the concept of “sterility” in this context is not understood as a single final stage of production, but rather as an integrated system of strict microbial contamination control throughout all manufacturing stages, starting from raw materials, passing through intermediate stages, and reaching final filling, using aseptic processing techniques and highly controlled environments. Accordingly, there are no “non-sterile” biological drugs in the final injectable product, but only “controlled contamination” during manufacturing stages (bioburden), which is maintained within very strict limits. Contamination is not expected to reach the final product due to a set of strict controls including fine filtration, clean rooms, continuous microbiological testing, and strict monitoring of all production stages, in addition to the fact that manufacturing conditions are often not suitable for the growth or survival of many microorganisms until the end of the process. In the case of microbial contamination during intermediate stages, it is controlled either by removal through filtration or reduction under strict operating conditions that prevent its proliferation or transfer to the final product. The determination of the number and type of microorganisms (quantity and type) is highly important, as it helps to understand the source of contamination and select the appropriate strategy for its control and elimination. Thus, the objective of this system is not only to achieve sterility as a final state, but to ensure a high level of confidence in the absence of contamination known as the Sterility Assurance Level (SAL), which represents an indicator of the degree of sterility assurance rather than the result of a single test. Accordingly, all qualitative and quantitative microbiological tests are used as tools for control and monitoring to ensure that the final injectable biological product meets sterility and therapeutic safety requirements for the patient. The importance of these tests lies in their role as a fundamental tool to ensure compliance with pharmacopoeial standards such as USP and EP, as well as in evaluating the effectiveness of cleaning and sterilization procedures within the manufacturing environment, and determining the suitability of raw materials before entering sensitive production stages, thereby contributing to the protection of the quality and safety of final biological products.

2. 5. 1. Bioburden Test

The Bioburden Test is considered one of the most important fundamental tests in microbiological quality control during the manufacturing of biological drugs, as it aims to measure the total number of viable microorganisms present on raw materials, intermediate stages, or on surfaces and equipment prior to final sterilization processes. This test does not aim to identify microbial species as much as it focuses on estimating the total microbial contamination level, which allows evaluation of the effectiveness of hygiene and manufacturing procedures within the production environment. This test is based on sampling from the product or surfaces, followed by inoculation into suitable culture media under defined incubation conditions (temperature and time), after which the growing bacterial or fungal colonies are counted and expressed in CFU (Colony Forming Units). The higher the number of CFUs, the weaker the microbial control in the manufacturing process, and vice versa. The importance of this test lies in its role as a fundamental preventive step, as it helps determine the contamination level before reaching the final sterilization stage, thereby reducing the risk of batch failure and ensuring the quality and safety of sensitive biological products such as vaccines, therapeutic proteins, and monoclonal antibodies. It is also used to evaluate the effectiveness of cleaning and sterilization procedures (Cleaning and Sterilization Validation) within production lines. Accordingly, the Bioburden Test is considered a vital indicator of manufacturing environment quality and forms a fundamental basis for subsequent microbiological tests. It plays a key monitoring role in ensuring the effectiveness of hygiene and sterilization procedures, reducing the risk of contaminant transfer to sensitive manufacturing stages, and thereby maintaining the quality and safety of biological drugs.

2. 5. 2. Microbial Enumeration Tests

Microbial Enumeration Tests are considered one of the essential tests in the microbiological quality control of biological and non-sterile drugs, and they aim to determine the total number of viable microorganisms present in raw materials, intermediate products, or within the production environment in manufacturing units. This type of testing includes three main measurements:

2. 5. 2. 1. Total Aerobic Microbial Count (TAMC)

The TAMC test is a microbiological test used to determine the total number of aerobic microorganisms capable of growing in the presence of oxygen within a sample, and it is considered an important indicator of the overall bacterial contamination level. The test is performed by taking a sample of the material, diluting it, and then inoculating it onto suitable culture media that allow the growth of aerobic bacteria, followed by an incubation step under specified conditions, after which the resulting colonies are counted and expressed in CFU (Colony Forming Units). It primarily aims to assess the level of microbial contamination in raw materials or intermediate products and is considered a control step directly related to the safety of the manufacturing process. This test does not focus on the type of microorganisms but rather on their number in order to control process quality and prevent contamination transfer to final stages. It focuses on monitoring microbial contamination during manufacturing stages prior to sterilization and is used as a process control tool.

2. 5. 2. 2. Total Yeast and Mold Count (TYMC)

The TYMC test aims to measure the total number of yeasts and fungi present in the sample, which are microorganisms that may lead to spoilage of pharmaceutical products or affect their chemical and physical stability. The sample is inoculated onto suitable culture media for fungal growth such as Sabouraud Dextrose Agar, then incubated at a lower temperature compared to bacteria and for a relatively longer period. After that, the resulting fungal colonies are counted. This test helps detect fungal contamination and ensure the quality and stability of biological drugs.

2. 5. 2. 3. Total Viable Count (TVC)

The TVC test is a comprehensive test aimed at determining the total number of viable microorganisms capable of growth in the sample, including bacteria, yeasts, and fungi, and it is considered a general indicator of the overall microbial load. This test is based on inoculating the sample onto suitable culture media, followed by incubation under conditions that allow the growth of different types of microorganisms, after which the resulting colonies are counted and expressed in CFU (Colony Forming Units). It is used to evaluate general microbiological cleanliness and ensure quality control in production processes.

2. 5. 2. 4. Most Probable Number (MPN / NPP)

The Most Probable Number (MPN / NPP) test is a statistical method used within microbial enumeration tests to estimate the number of viable microorganisms in a sample, especially when their number is very low or when they are not easily able to grow on solid media. This test is based on preparing a series of serial dilutions of the sample, then inoculating each dilution into liquid culture media. The tubes are then incubated under conditions suitable for microbial growth. The number of tubes showing microbial growth (turbidity, gas production, or color change) is recorded, and specific statistical tables are used to estimate the most probable number of microorganisms in the sample. The importance of this test lies in its ability to provide an approximate estimation of microbial load when direct counting methods are difficult to apply. It is also used in specific cases such as water analysis or samples with low microbial density, making it a complementary tool to conventional enumeration methods in the microbiological quality control of biological drugs.

2. 5. 3. Sterility Test

The Sterility Test is considered one of the most important final microbiological tests in the quality control of biological drugs intended for parenteral administration, as it aims to ensure that the final product is completely free from any viable microorganisms such as bacteria, fungi, and yeasts, in accordance with the requirements of international pharmacopoeias such as USP and EP. This test is applied to products that are required to be fully sterile and is not considered a method of sterilizing the product, but rather a confirmatory test that verifies the achievement of sterility after the completion of all manufacturing stages. This test is based on incubating the sample in suitable culture media for a defined period under conditions that allow the growth of any potential microbial contamination, followed by observation of microbial growth. The absence of growth indicates compliance with sterility requirements. The Sterility Test is performed using two main methods:

2. 5. 3. 1. Membrane Filtration Method

The Membrane Filtration Method is one of the most widely used methods in sterility testing, especially for liquid or filterable biological products. This method is based on passing

a defined volume of the sample through a very fine membrane filter, usually with a pore size of about 0.45 μm or smaller, where this membrane retains all potential microorganisms such as bacteria, yeasts, and fungi, while allowing the small pharmaceutical components to pass through. After filtration, the membrane is washed to remove any residues that may affect microbial growth, then it is divided and placed directly into suitable culture media, such as a medium that promotes the growth of aerobic bacteria and another medium designed for anaerobic organisms. The media are then incubated for a standard period under specified temperature conditions, and any microbial growth is monitored. If no growth appears during the incubation period, the product is considered compliant with sterility requirements. This method is characterized by its high capacity to test large sample volumes, which increases its sensitivity in detecting microbial contamination even when present in very low numbers.

2. 5. 3. 2. Direct Inoculation Method

The Direct Inoculation Method is used as an alternative to the membrane filtration method in cases where the product cannot be filtered, such as viscous or oily preparations or those containing substances that may block the membrane pores. This method is based on the direct addition of a defined quantity of the sample into sterile and suitable culture media for microbial growth, whether media for aerobic bacteria, anaerobic bacteria, or fungi. These media are then incubated under standard conditions in terms of temperature and time, after which any changes indicating microbial growth, such as turbidity or colony formation, are observed. If no microbial growth appears during the defined incubation period, the product is considered to meet sterility test requirements. Although this method is less sensitive compared to membrane filtration, it is essential for certain formulations whose physical properties do not allow filtration, making it an integral part of the sterility assurance system in the pharmaceutical industry for biological drugs.

2. 5. 4. Specified Microorganisms Test

The Specified Microorganisms Test is considered one of the essential qualitative microbiological tests in drug quality control, particularly for non-sterile products or raw materials and intermediate stages in the manufacturing of biological drugs. It aims to detect the presence of specific types of microorganisms that are considered an indicator of serious

Chapter II Quality Control in Biopharmaceutical Manufacturing

contamination or poor manufacturing conditions. Unlike microbial enumeration tests, which focus on the total count, this qualitative test focuses on the presence or absence of highly hazardous microorganisms, even in very small numbers, because their presence is not acceptable in pharmaceutical products. This test includes the detection of a group of microorganisms of high regulatory importance in the pharmaceutical industry as follows:

- ***Escherichia coli***

- o Type: Facultative aerobic bacteria

- o Source: Fecal contamination, untreated water, or poor hygiene of raw materials during preparation and manufacturing stages.

- ***Salmonella spp.***

- o Type: Pathogenic facultative aerobic bacteria

- o Source: Environmental contamination, contaminated raw materials of animal or food origin, or manufacturing water.

- ***Staphylococcus aureus***

- o Type: Gram-positive aerobic bacteria

- o Source: Human skin or respiratory tract of personnel within the manufacturing environment due to contact or airborne droplets.

- ***Pseudomonas aeruginosa***

- o Type: Gram-negative aerobic bacteria

- o Source: Moist environments within production lines such as non-sterile water or cleaning systems (Water systems and humid environments).

- ***Candida albicans***

- o Type: Yeast (unicellular fungi)

- o Source: Human skin and mucous membranes or environmental contamination in non-fully sterile manufacturing areas.

- *Clostridium spp.*

- o Type: Obligate anaerobic bacteria

- o Source: Soil, dust, or contaminated raw materials containing spores capable of surviving harsh conditions, and may appear in cases of weak sterilization or cleaning control within the production environment.

This test is based on selective culture techniques using specific media designed for each microorganism, where the growth of the target organism is promoted while inhibiting others through selective agents or specific incubation conditions. The results are then interpreted based on the presence or absence of characteristic growth for each organism. The importance of this test lies in its critical role in ensuring the safety of pharmaceutical materials and preventing the entry of highly hazardous microorganisms into final production stages or to the end user, making it an essential component of pharmaceutical quality systems and compliance with USP and EP standards.

2. 5. 5. Anaerobic Bacteria Detection Test

The Anaerobic Bacteria Detection Test is considered an important microbiological test in the quality control of biological drugs, particularly in intermediate stages and raw materials. It aims to detect the presence of bacteria that are unable to grow in the presence of oxygen, which may pose a significant risk if they reach the final product or become established in the manufacturing environment. This test relies on providing oxygen-free conditions that allow the growth of these microorganisms only if they are present in the sample, making it a highly important qualitative test for detecting hidden contamination that may not appear in conventional aerobic tests. The test is performed using Fluid Thioglycollate Medium (FTM), a liquid medium specifically designed to reduce oxygen content within the medium and provide a suitable environment for the growth of anaerobic bacteria. The sample is inoculated by injection or direct addition into the broth medium rather than surface plating, to ensure that microorganisms remain in a low-oxygen environment. The tubes are then

incubated under standard temperature conditions, with monitoring for any signs of growth such as turbidity, sediment formation, or color change. Any observed growth is considered evidence of anaerobic bacterial contamination in the sample. The importance of this test lies in its ability to detect microorganisms that are not identified in aerobic tests, such as certain species of *Clostridium* spp., which are obligate anaerobic spore-forming bacteria capable of surviving harsh environmental conditions. Therefore, this test is used as an essential part of the microbiological quality assurance system to ensure that products or materials are free from anaerobic contamination that may affect the safety of biological preparations.

2. 5. 6. Bacterial Endotoxin Test

The Endotoxin Test is considered one of the essential biological microbiology tests in the quality control of biological drugs, especially injectable or intravenous preparations. It aims to detect the presence of endotoxins, which are toxic components released from the cell wall of Gram-negative bacteria upon lysis or death, particularly bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. This test differs from the sterility test because it does not detect the presence of viable microorganisms themselves, but rather detects toxins that may remain even after bacterial elimination, which makes it highly important for ensuring the biological safety of the final product. The test is based on the Limulus Amebocyte Lysate (LAL) test, a substance extracted from the blood of a marine organism known as the horseshoe crab. Its blood contains special cells called amebocytes, which are highly sensitive to bacterial endotoxins and react with them, producing a clotting reaction or measurable changes in the laboratory. This substance is used as a biological reagent for highly accurate endotoxin detection.

The methods of this test include:

- Gel-Clot Method, which depends on gel formation in the presence of endotoxins.
- Turbidimetric Method, which measures the turbidity produced by the reaction.
- Chromogenic Method, which relies on color change resulting from an enzymatic reaction.

The importance of this test lies in its essential role in ensuring that biological products, especially injectable ones, do not cause fever reactions or toxic shock in patients, as even very

small amounts of endotoxins can lead to severe effects when entering the bloodstream. Therefore, this test is a fundamental component of quality assurance and safety systems in the biological pharmaceutical industry.

2. 5. 7. Mycoplasma Detection Test

The Mycoplasma Detection Test is considered one of the advanced and essential microbiological tests in the quality control of biological drugs, especially those produced using cell culture techniques. It aims to detect contamination caused by microorganisms known as mycoplasma, which are a unique type of bacteria characterized by the absence of a cell wall, making them extremely small in size and capable of passing through conventional sterilizing filters (such as 0.22 μm), and they are not easily detected using traditional culture methods. Their presence may lead to serious alterations in the cells used in production, thereby affecting the quality and efficacy of the final biological product.

-Mycoplasma detection is performed using several methods, the most important of which are:

- o **Culture Method**, which is based on growing mycoplasma in specific media and is considered the reference method despite its slow turnaround time.
- o **Polymerase Chain Reaction (PCR Method)**, which is considered one of the fastest and most sensitive methods, as it detects the genetic material of mycoplasma.
- o **Enzyme-Linked Immunosorbent Assay (ELISA Method)**, which is based on detecting specific components or antigens of these microorganisms. The appropriate method is selected according to the nature of the sample and the required sensitivity level.

The importance of this test lies in the fact that mycoplasma contamination usually does not cause visible turbidity or typical growth signs, making it a hidden and dangerous type of contamination, especially in biological industries based on living cells. Therefore, its detection is considered a critical step to ensure the safety and quality of biological drugs and to prevent any negative effects on the final product or patient safety.

2. 5. 8. Viral Detection Test

The Viral Detection Test is considered one of the advanced biological safety tests in the quality control of biological drugs, especially those produced using cell culture or

materials of animal origin. It aims to detect the presence of viruses that may contaminate the product during manufacturing stages. Viruses are non-cellular microorganisms that differ from bacteria, as they cannot grow in conventional culture media and require living cells for replication. They are also extremely small and capable of passing through standard sterilization filters, which makes their detection more complex and requires specialized techniques.

Virus detection is performed using several advanced methods, the most important of which are:

- o **Polymerase Chain Reaction (PCR / Real-Time PCR):** This method is based on amplifying the viral genetic material (DNA or RNA) if present in the sample, allowing detection of very small quantities with high sensitivity and accuracy. Real-Time PCR also enables real-time monitoring of the reaction and semi-quantitative measurement of viral load.
- o **Enzyme-Linked Immunosorbent Assay (ELISA):** This method is based on the interaction between viral antigens and specific antibodies, where viral proteins are detected through a colorimetric reaction that can be measured. It is characterized by its ability to analyze a large number of samples rapidly.
- o **Cell Culture Assay:** This method is used to detect viruses capable of infecting cells, where the sample is inoculated into suitable living cells and then observed for changes such as deformation or cytopathic effects. This method is important for assessing viral replication capacity and biological impact, although it is slower compared to molecular techniques.

The importance of this test lies in the fact that viral contamination may lead to serious health risks for patients and may also affect the safety and efficacy of biological products, especially those based on living cell systems. Therefore, this test is an essential part of the biological quality assurance system and is used to ensure that biological products are free from viral contamination before being released for use.

2. 5. 9. Rapid Microbiological Methods (RMMs)

Rapid Microbiological Methods are considered modern techniques used in the microbiological quality control of biological drugs. They aim to detect microorganisms faster compared to conventional culture-based methods, which may require several days. These methods rely on advanced physical, chemical, and biological techniques that allow direct or indirect detection of microorganisms or their metabolic activity, thereby accelerating decision-making during manufacturing and reducing product release time while maintaining accuracy and reliability of results.

The most important of these methods include:

- ❖ **Flow Cytometry:** This technique is based on passing microbial cells in a fluid stream through a laser beam, allowing rapid counting and analysis of their properties such as size, structure, and viability. Fluorescent dyes are often used to distinguish between live and dead cells.
- ❖ **Fluorescent Staining Methods:** These methods rely on the use of specific dyes that bind to particular cellular components such as DNA or cell membranes. These dyes emit fluorescence when exposed to light, allowing rapid detection of microorganisms even without cultivation.
- ❖ **ATP Bioluminescence Technique:** This method is based on measuring the amount of adenosine triphosphate (ATP) present in living cells, as ATP is a direct indicator of biological activity. It is detected through an enzymatic reaction that produces measurable light, where higher light intensity indicates a higher number of viable microorganisms.

The importance of these methods lies in their ability to provide rapid and accurate results compared to conventional techniques, enabling early detection of microbial contamination and immediate corrective actions, especially in sensitive biological industries that require strict quality control. However, these methods require thorough validation before implementation to ensure compliance with pharmacopoeial standards such as USP and EP.

2. 6. Linking Test Results to Manufacturing Decisions

Test results in the microbiological manufacturing of biological drugs are not merely analytical data or reports; they are directly used to make critical decisions regarding the fate of production batches. Results from all tests such as microbial enumeration (TAMC and TYMC), sterility testing, and endotoxin testing are compared with predefined acceptance criteria established by international regulatory authorities.

If the results fall within acceptable limits, a Batch Release decision is issued, meaning the product is safe and can be marketed. However, if results show excessive microbial counts, presence of pathogenic microorganisms, or elevated endotoxin levels, the decision is either batch rejection or reprocessing depending on the nature and correctability of the issue.

Interpretation of results is not limited to acceptance or rejection; it is also used to identify the root causes of deviations in the manufacturing process, such as:

- Raw material contamination.
- Defects in the manufacturing environment.
- Ineffective equipment sterilization.
- Human errors or operational procedure deviations.

Thus, this stage represents a link between laboratory analysis and manufacturing management, where data is translated into practical decisions aimed at improving production quality and preventing recurrence of errors.

2. 6. 1. International Regulatory Systems and Authorities

Microbiological test results in biological drug manufacturing are not merely analytical data; they represent a scientific and regulatory basis for final decisions regarding batch acceptance or rejection. This is achieved by comparing results with acceptance criteria defined by international regulatory pharmacopoeial authorities to ensure the quality, safety, and efficacy of pharmaceutical products before market release.

The quality assurance system in this context is divided into two interconnected levels:

2. 6. 1. 1. Quality Systems / Quality Standards

These are regulatory systems or operational frameworks applied within manufacturing facilities and laboratories to ensure correct and documented implementation of standards, ensuring controlled and compliant workflows. They include:

- **Good Manufacturing Practices (GMP):** A system that defines how drugs must be manufactured safely and systematically, focusing on hygiene, sterilization, process documentation, and contamination prevention (e.g., cleanroom design and personnel behavior).
- **Good Laboratory Practices (GLP):** A system ensuring that laboratory testing is performed accurately, reliably, and reproducibly (e.g., proper execution of TAMC and sterility tests).
- **International Organization for Standardization (ISO):** Provides global technical standards, such as cleanroom standards (ISO 14644) and microbial contamination monitoring (ISO 14698), e.g., permissible airborne particle limits.

These systems do not constitute legally binding regulations for countries but are used to standardize and control industrial quality practices within organizations.

2. 6. 1. 2. Regulatory Authorities / Organizations

These are official national or international bodies that establish laws, standards, and microbiological limits that pharmaceutical products must comply with before marketing, and they oversee their enforcement. They are the ultimate reference for drug approval and include:

- **World Health Organization (WHO):** Provides global guidelines for ensuring drug quality and safety, particularly important for developing countries.
- **United States Pharmacopeia (USP):** A key reference in the USA and globally, defining microbiological tests (sterility testing, TAMC/TYMC limits, endotoxin limits) and acceptance criteria.

- **European Pharmacopoeia (EP):** The official European reference, similar to USP but more detailed in certain areas, defining quality standards and approved tests.
- **International Council for Harmonisation (ICH):** Aims to harmonize standards between Europe, the USA, and Japan, especially for biological drugs, and provides unified guidelines for drug development.

These authorities define the actual acceptance criteria against which test results are evaluated.

3. 6. 3. Key Acceptance Criteria in Biological Drugs

❖ Sterile Products

→ Must show 0 CFU / No microbial growth in sterility testing.

❖ Non-Sterile Products

→ Limited microbial load is allowed within defined limits for:

- TAMC (Total Aerobic Microbial Count)
- TYMC (Total Yeast and Mold Count)

depending on product type and route of administration.

❖ Bacterial Endotoxins

→ Must be below acceptable limits, typically:

- ≤ 5 EU/kg depending on product type
- In some sensitive intravenous products down to 0.25 EU/mL

❖ Specified Microorganisms

→ Must be completely absent, such as:

- *Escherichia coli*

- Salmonella spp.
- Pseudomonas aeruginosa

2. 6. 4. Manufacturing Decision Making

Based on these criteria, the final decision is made:

- **Batch Release:** if all results are within acceptable limits.
- **Reprocessing/Rejection:** if any limit is exceeded or unacceptable microorganisms are detected.

In addition, these results are not only used for final decisions but also support quality monitoring and manufacturing control through regulatory compliance, deviation investigations, and tracing contamination sources throughout production stages.

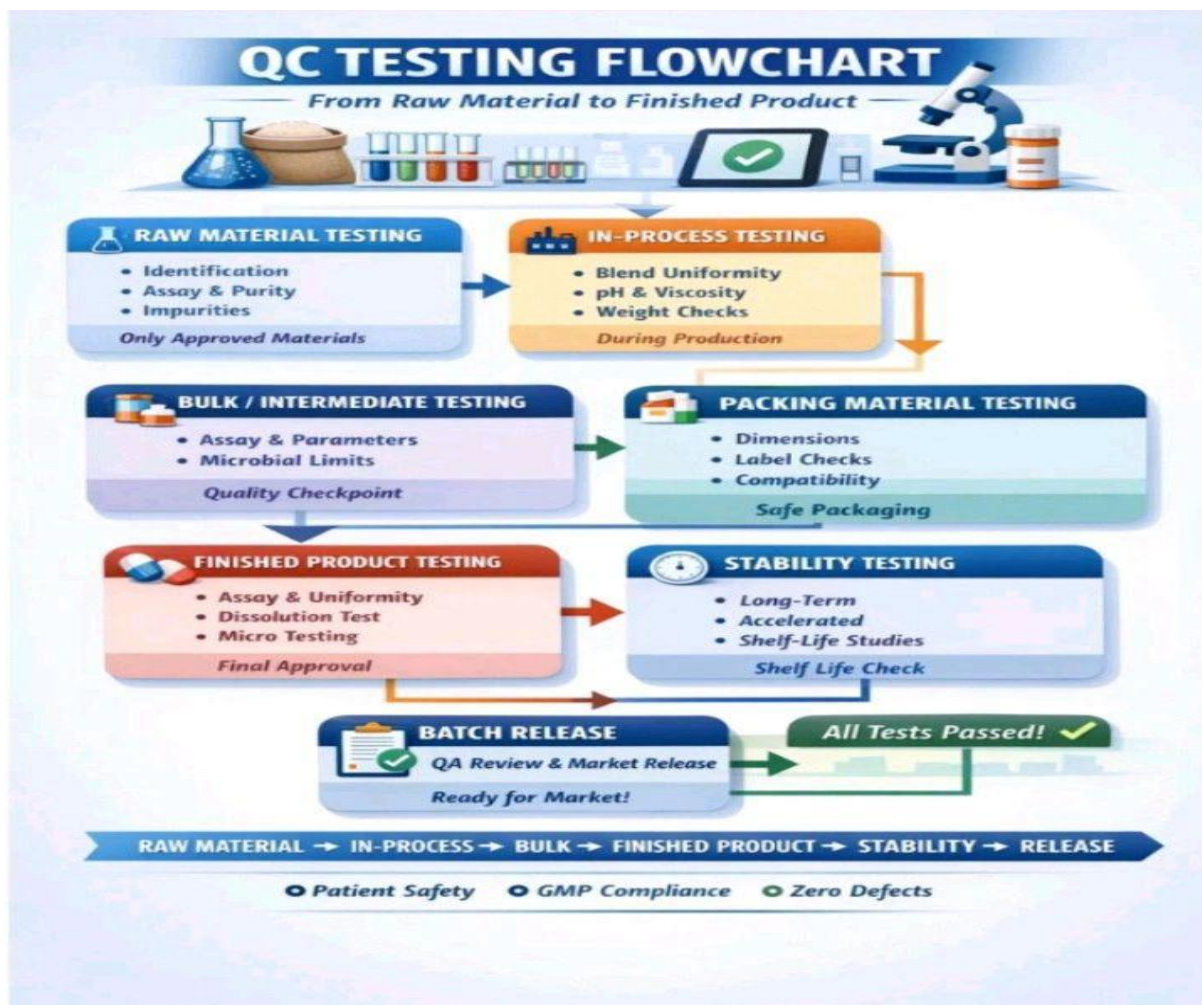


Figure 16: Stages of quality control in the manufacturing of biological drugs, starting from raw materials up to the final product ready for marketing.

3. Toxicity Monitoring Techniques

Toxicity Monitoring Techniques are considered vital stages in the evaluation of the quality and safety of biological drugs, as they aim to detect any potential harmful effects that may occur on cells, tissues, or body systems as a result of exposure to pharmaceutical preparations. These techniques are of particular importance in biological drugs due to their complex composition and high sensitivity, making toxicity assessment an essential requirement to ensure safe therapeutic use. These techniques include the study of different types of toxicity such as cytotoxicity, which measures the effect of a substance on cell viability and growth, immunotoxicity, which evaluates its effect on the immune system either through suppression or excessive stimulation, and systemic toxicity, which studies the effect

of the drug on vital organ functions after entering the bloodstream. These evaluations rely on both *in vivo* and *in vitro* laboratory tests using cellular models, animal models, or advanced cell-based systems, in order to determine safe doses and assess the dose–response relationship. These techniques contribute to reducing potential risks and improving the reliability of biological drugs before reaching clinical stages.



Figure 17: General stages of toxicity monitoring in biological drugs, starting from *in vitro* laboratory testing on cells, then moving to *in vivo* animal models, before reaching clinical evaluations, in order to ensure drug safety and efficacy prior to human use.

3.1. Definition of Toxicity Monitoring Techniques

Toxicity Monitoring Techniques are defined as a set of scientific methods and experiments used to evaluate the potential harmful effects of pharmaceutical substances, especially biological drugs, on cells, tissues, and the living organism in general, with the aim of ensuring their safety prior to therapeutic use. These techniques are based on studying the response of different biological systems when exposed to the pharmaceutical substance through *in vitro* tests using cellular models, or *in vivo* tests using animal models, where functional or structural changes resulting from exposure are analyzed. These evaluations

include the measurement of multiple indicators such as cell viability, cell death rate, and oxidative stress, in addition to studying the dose–response relationship to determine safe usage limits. These techniques are of particular importance in biological drugs due to their complex structure and potential interaction with the immune system, which requires precise and sensitive methods for early detection of possible toxic effects, thereby ensuring the quality and efficacy of the drug product and patient safety.

3.2. Importance of Toxicity Monitoring Techniques

Toxicity Monitoring Techniques are of great importance in the development and manufacturing of biological drugs, as they represent an essential step to ensure product safety before human use. Due to the complex nature of these drugs and their reliance on biological components, they may produce unexpected effects on cells or the immune system, which necessitates precise evaluation of toxicity levels at different stages of development. The importance of these techniques lies in their ability to detect potential harmful effects early, whether at the cellular level (cytotoxic effects), tissue level, or functional level, allowing for modification of the formulation or adjustment of dosages before reaching clinical stages. They also contribute to determining the dose–response relationship and identifying the safe dose and maximum tolerated dose, as well as assessing risks associated with long-term use. Furthermore, these techniques play an important role in compliance with international regulatory requirements, as such studies are required by regulatory authorities to ensure the quality and safety of drugs. Therefore, toxicity monitoring is not only about patient protection, but also a key factor in improving drug efficacy and ensuring successful clinical and marketing outcomes.

3.3. Types of Toxicity in Biopharmaceuticals

Toxicity in biological drugs is classified into several main types according to the nature of the effect exerted by the pharmaceutical substance within the body. This classification is essential for understanding mechanisms of toxic action and accurately defining evaluation methods. Since biological drugs interact directly with biological systems, especially cells and the immune system, they may induce different responses ranging from direct cellular effects to immune disturbances or systemic effects involving multiple body

organs. Therefore, toxicity is divided into three main categories: cytotoxicity, which affects cell survival and function; immunotoxicity, which relates to drug effects on the immune system either through suppression or excessive stimulation; and systemic toxicity, which reflects the overall effect of the drug on vital organ functions after distribution throughout the body. This classification helps guide appropriate testing strategies for each toxicity type, as evaluation methods and indicators vary depending on the nature of the effect. It also enables identification of potential risk sources during drug development and manufacturing stages. Thus, understanding toxicity types is a fundamental step in designing effective biological safety monitoring strategies and ensuring that biological products do not produce unacceptable harmful effects during therapeutic use.

3.3.1. Cytotoxicity

Cytotoxicity is considered one of the most important forms of toxicity evaluated in biological drugs, as it refers to the ability of a pharmaceutical substance to cause direct damage to living cells, leading to inhibition of their growth, disruption of their functions, or even cell death. The study of this type of toxicity is of great importance because cells are the basic unit of any biological system; therefore, any negative effect on them may extend to tissues and organs as a whole. Cytotoxicity is usually assessed using in vitro tests through the culture of living cells and exposing them to different concentrations of the drug, followed by measuring indicators such as cell viability, cell proliferation, and programmed cell death (apoptosis) or necrosis. These assays rely on several techniques such as the MTT assay, which measures cellular metabolic activity as an indicator of viability; the LDH release assay, which detects cell membrane damage by measuring lactate dehydrogenase released from damaged cells; and fluorescent staining techniques that distinguish between live and dead cells.

The importance of these studies lies in determining the toxic concentrations of the drug and evaluating the dose–response relationship, which helps establish safe usage limits and reduce potential risks before proceeding to animal or clinical studies.

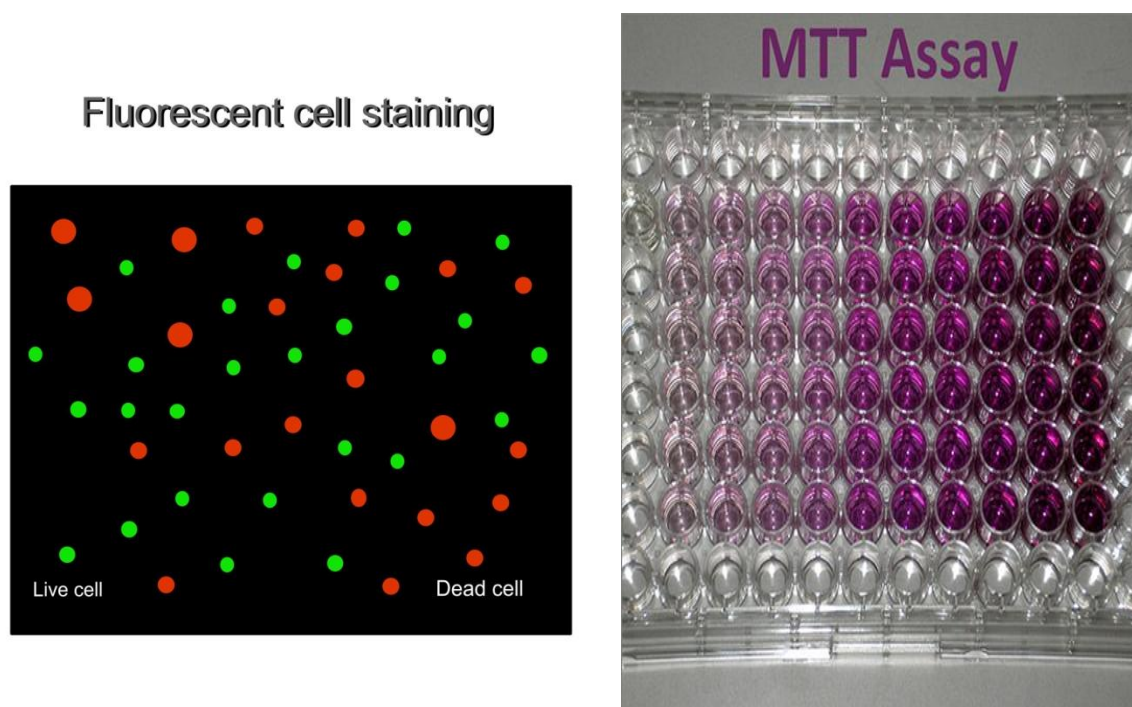


Figure 18: Tests of the effect of pharmaceutical substances on cells, where the percentage of living cells versus dead cells is measured to determine the degree of toxicity of the substance on cell growth and survival.

3.3.2. Immunotoxicity

Immunotoxicity is defined as the harmful effects that biological drugs may exert on the immune system, either through immunosuppression, excessive immune stimulation, or disruption of the normal balance of immune responses. This type of toxicity is of particular importance in biological drugs because these products are often proteins or complex molecules capable of directly interacting with components of the immune system, which may lead to undesirable responses such as hypersensitivity reactions or the formation of anti-drug antibodies. Immunotoxicity is evaluated using a combination of *in vitro* and *in vivo* tests, where parameters such as immune cell activity, cytokine levels, lymphocyte proliferation, and antibody production are measured. Among the most commonly used techniques in this field are ELISA assays for quantifying cytokines or antibodies, flow cytometry for analyzing immune cell populations and proportions, as well as animal models to assess the overall immune response. The importance of studying immunotoxicity lies in its ability to detect early immune disturbances that may lead to loss of drug efficacy or serious adverse effects

such as hypersensitivity or immunosuppression, making it a fundamental step in evaluating the safety and efficacy of biological drugs prior to therapeutic use.

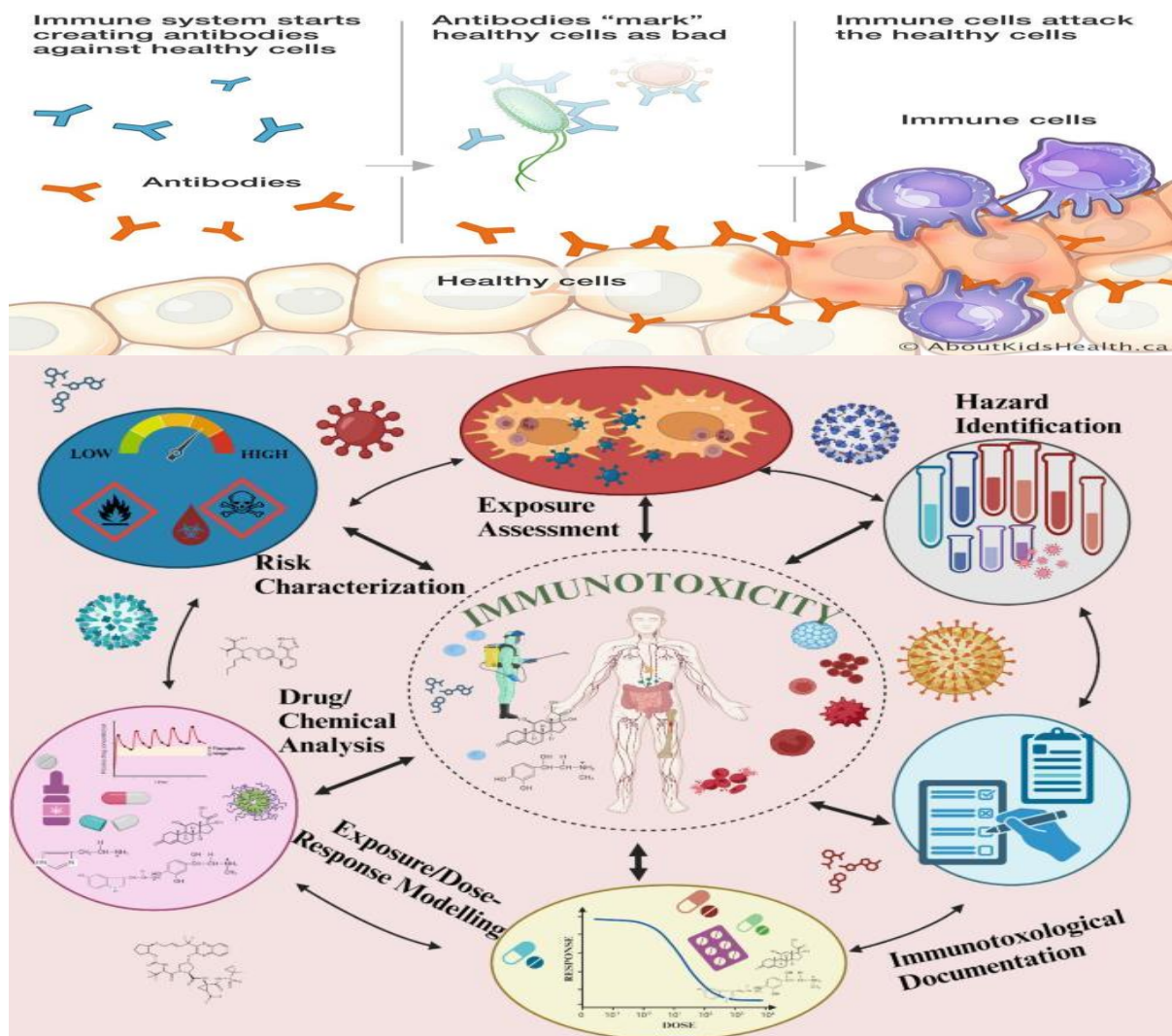


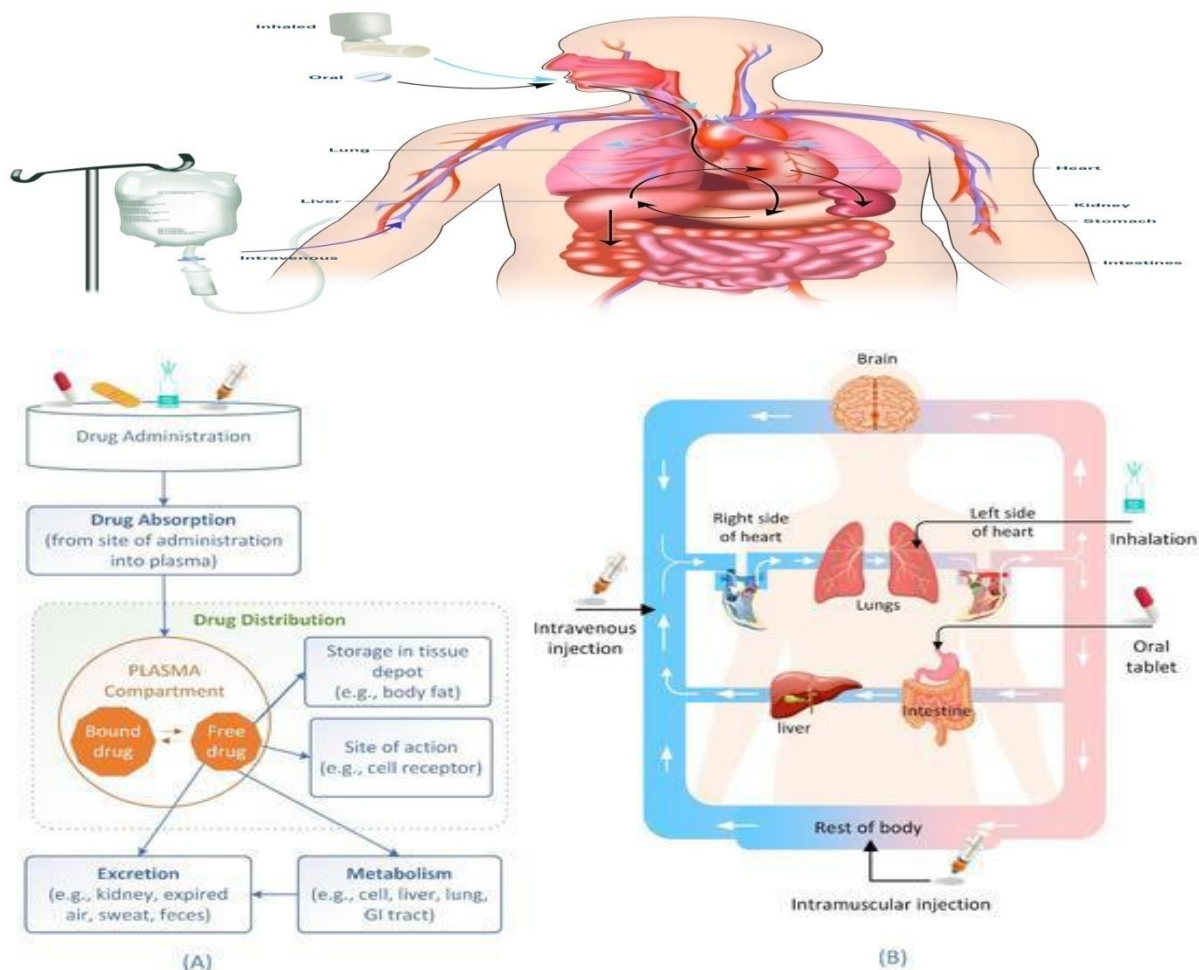
Figure 19: The effect of a drug on the immune system, either through immunosuppression or excessive immune activation (hyperactivation), which may lead to harmful immune responses.

3.3.3. Systemic Toxicity

Systemic toxicity is defined as the harmful effects induced by biological drugs at the level of the whole body after the pharmaceutical substance enters the bloodstream and distributes across different organs and tissues. Unlike cytotoxicity or immunotoxicity, this type of toxicity is not limited to a specific target, but may involve multiple vital physiological

Chapter II *Quality Control in Biopharmaceutical Manufacturing*

functions such as the liver, kidneys, heart, and nervous system. Therefore, systemic toxicity is considered one of the most important parameters evaluated to ensure that the drug does not cause widespread functional disturbances when administered at therapeutic doses or under repeated exposure. Systemic toxicity is assessed through *in vivo* studies using appropriate animal models, where behavioral, functional, and physiological changes are monitored, along with the measurement of biomarkers such as liver enzymes, renal function tests, and hematological parameters. Additional assessments include body weight monitoring, general activity, and survival rate. Repeated dose toxicity studies are also used to evaluate the effects of continuous exposure to the drug and to determine the No Observed Adverse Effect Level (NOAEL). The importance of this toxicity type lies in providing a comprehensive picture of drug safety within the organism. It helps determine safe human doses and is a fundamental requirement of regulatory authorities to ensure that no severe systemic effects occur that could endanger patient life or impair vital organ functions.



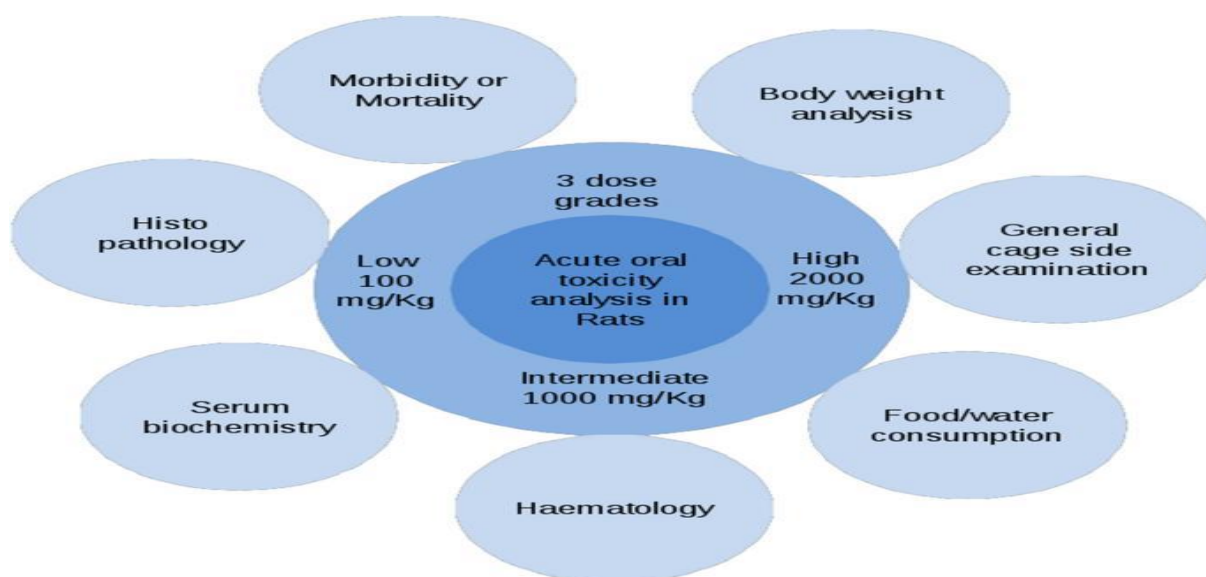


Figure 20: The effect of toxic substances on vital organs such as the liver, kidneys, and heart, and it is usually studied through animal experiments to evaluate the overall damage of the drug within the body.

3.4. *In vitro* & *In vivo* Toxicity Assays

Toxicity assays are considered one of the fundamental pillars in evaluating the safety of biological drugs, as they aim to study the effect of a pharmaceutical substance on biological systems at different stages of preclinical development. These assays are divided into two main types: *in vitro* assays, which are performed on isolated cells or tissues under controlled laboratory conditions, and *in vivo* assays, which are conducted on animal models to study the overall effect of the drug within a living organism. This integration of both approaches allows a comprehensive evaluation that combines cellular precision with physiological relevance. *In vitro* assays rely on advanced cell-based systems such as cell culture, where cells are exposed to different concentrations of the biological drug and toxicity indicators are measured, including cell viability, cell proliferation rate, and membrane integrity, in addition to molecular markers related to stress or cell death. These assays are characterized by being rapid, cost-effective, and highly suitable for analyzing mechanisms of action at the cellular level with high precision. *In vivo* assays, on the other hand, are used to evaluate the overall effects of the drug on the entire organism, including biodistribution and effects on vital organs. These studies involve monitoring clinical signs such as behavior, body weight, survival rate, as well as biochemical analyses such as liver and kidney function tests,

blood parameters, and histopathological examination. These assays are essential for determining the dose–response relationship and assessing long-term safety, especially for biological drugs that may induce complex interactions within the body. The combination of *in vitro* and *in vivo* assays is essential to obtain a comprehensive and accurate toxicity assessment, where *in vitro* results help explain underlying mechanisms, while *in vivo* results confirm actual safety within the living organism, thereby reducing risks and ensuring the highest levels of drug safety.

3.5. Toxicity Evaluation Parameters

Toxicity evaluation parameters are a set of scientific measurements and criteria used to determine the degree of toxic effects of biological drugs on biological systems, whether at the cellular, tissue, or whole-organism level. These parameters are essential tools for converting experimental results into interpretable data that support drug safety assessment, as they are used to analyze dose–response relationships and define safe usage limits. These parameters include multiple aspects such as cellular, biochemical, immunological, and functional indicators, allowing a comprehensive and accurate toxicity evaluation. Key parameters include cell viability, which reflects the percentage of living cells after exposure to the drug, and cell death rate, which includes apoptosis and necrosis. Liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are used as indicators of liver integrity, while renal function markers such as creatinine and urea reflect kidney excretory performance. Other parameters include cytokine levels to assess immune response, hematological parameters such as white blood cell, red blood cell, and platelet counts, as well as behavioral indices and body weight changes as general indicators of the organism’s health status. The importance of these parameters lies in their ability to provide a quantitative and qualitative framework for toxicity assessment, enabling researchers to compare results across studies and accurately determine drug safety levels. They are also used in decision-making regarding progression to clinical stages or the need for formulation or dose adjustment to ensure maximum safety.

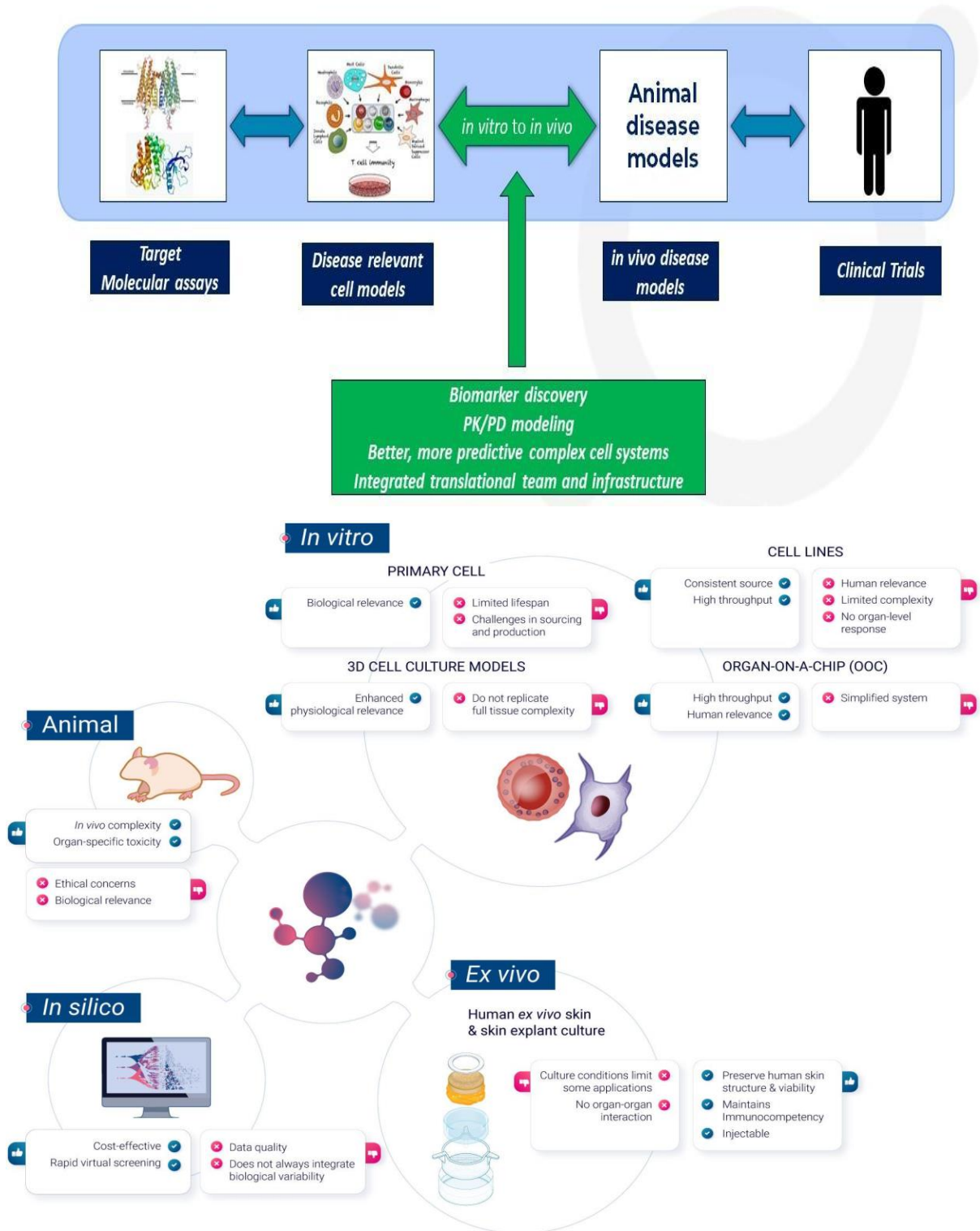


Figure 21: The integration between *in vitro* laboratory tests and *in vivo* biological tests. Cellular results are used to understand detailed mechanisms, while animal studies are used to confirm physiological safety, in order to provide a comprehensive toxicity assessment and reduce risks prior to clinical use.

Conclusion

Physicochemical, microbiological, and toxicity monitoring techniques are considered fundamental pillars within the pharmaceutical manufacturing control system, as they are used in an integrated manner to ensure the overall quality of the drug product throughout all stages of production. Physicochemical techniques allow the verification of identity, purity, concentration, and the physical and chemical stability of both the active substance and the final product, ensuring compliance with pharmacopoeial specifications and achieving the required therapeutic efficacy. Microbiological techniques focus on detecting bacterial and fungal contamination and confirming the absence of microorganisms and endotoxins in pharmaceutical preparations, especially sterile products, thereby ensuring safe use and protecting patients from infection risks. Toxicity techniques aim to evaluate the potential adverse effects of the drug in both the short and long term, through the study of acute and chronic toxicity, effects on vital organs, and the potential to induce mutagenic or carcinogenic effects. Thus, the integration of these techniques ensures the quality, safety, and efficacy of pharmaceutical products in accordance with Good Manufacturing Practices (GMP) and international pharmacopoeial standards.

Immunostaining
and
Immunodetection
Technique

III. Immunostaining and Immunodetection Techniques

Immunostaining and immunodetection techniques are among the most precise analytical tools in biopharmaceutical sciences. They are widely used in the study and characterization of biomolecules within biological drugs and cellular systems, as well as in monitoring and analyzing biological components during the manufacturing stages of these drugs. These techniques are based on the specific interaction between an antigen and an antibody, which allows the detection of proteins and biomolecules, as well as their localization and concentration with high precision in different samples. Therefore, they are essential for understanding and monitoring biological processes related to the production, development, and quality assurance of biological drugs. Immunostaining techniques include immunofluorescence, which relies on fluorescent dyes linked to antibodies that produce light signals upon detection, and immunohistochemistry, which uses enzymes or chromogenic substrates to visualize antigen distribution within tissues. Immunodetection techniques include ELISA (Enzyme-Linked Immunosorbent Assay), which is based on an enzymatic reaction producing a colorimetric signal proportional to the amount of the target substance; Western Blot, which is used to identify and separate proteins according to molecular weight and detect them using specific antibodies; and flow cytometry, which enables rapid single-cell analysis and characterization of immunological properties with high accuracy. These techniques are essential tools in the characterization of biological drugs, assessment of their efficacy, and assurance of their quality before and during production and development stages. Immunostaining aims to visually localize the target molecule within cells or tissues using dyes or fluorescent signals, while immunodetection aims to detect the presence or quantify the target molecule using analytical techniques such as enzymes, chemical or fluorescent signals.

1. Definition of Immunostaining and Immunodetection Techniques

Immunostaining and immunodetection techniques are defined as advanced laboratory methods based on the specific interaction between an antigen and an antibody to detect biological molecules, determine their localization, or quantify their concentration within cells, tissues, or biological fluids. These techniques rely on the selective binding of antibodies to their biological targets, followed by visualization of this binding using detection systems such as enzymes, fluorescent dyes, or optical signals, allowing the interaction to be observed

visually or measured instrumentally. They are used to identify the localization of proteins within cells, detect specific antigens in biological samples, or measure concentrations of proteins associated with drug efficacy or toxicity, thereby enabling precise qualitative and quantitative analysis of biological components.

2. Importance of Immunostaining and Immunodetection Techniques in Biopharmaceutical Manufacturing

Immunostaining and immunodetection techniques are of great importance in the manufacturing of biological drugs, as they are used as essential tools in quality control and in identity and purity testing. This is achieved through their ability to analyze proteins and biomolecules with high precision and to distinguish between similar molecules during both development stages and quality monitoring. These techniques also contribute to understanding the mechanism of action of the drug and evaluating its stability and purity, in addition to ensuring that the product complies with the required biological specifications according to pharmaceutical manufacturing standards. They help confirm that the produced therapeutic proteins within cells are the correct proteins and in the appropriate quantity. They are also used to detect any deviations in protein expression or the presence of unwanted contaminants, as well as to assess cellular response to the drug and the stability of the bioproduction process. Furthermore, they are applied to monitor production stages in cell culture systems to ensure that cells are producing the active substance correctly and consistently. In addition, these techniques support industrial quality systems such as HACCP (Hazard Analysis and Critical Control Points) by monitoring critical control points in the manufacturing process and detecting errors at an early stage before the product reaches the final phase, thereby ensuring the safety and efficacy of the final biological drug product.

3. Principle of Antigen–Antibody Reaction

The principle of the antigen–antibody reaction is based on the specific and precise binding between an antigen and an antibody, where the antibody recognizes a specific region on the antigen known as the epitope and binds to it selectively without interacting with other molecules. In the context of biopharmaceutical manufacturing control, the antigen may be the biologically active substance itself, such as therapeutic proteins produced through genetic engineering techniques (e.g., monoclonal antibodies, protein hormones, and therapeutic

enzymes). It may also refer to unwanted molecules targeted during monitoring, such as host cell protein residues, process-related protein impurities, residual DNA from production cells, or misfolded protein aggregates. Additionally, it may include microbial contamination agents such as bacteria, viruses, or fungi when specific antibodies are designed to detect them. This binding occurs through non-covalent forces such as hydrogen bonds, electrostatic interactions, and van der Waals forces, leading to the formation of a relatively stable but reversible immune complex under certain conditions. This property is exploited in immunological techniques for the accurate detection of the biological active substance or associated impurities and contaminants during production and quality control stages. It enables the evaluation of batch quality and supports decisions related to product acceptance, rejection, or reprocessing. However, these techniques are primarily used as tools for detection, monitoring, and analysis, and not for direct removal of contaminants, which is instead achieved through manufacturing purification steps such as filtration, chromatography, and sterilization. This reaction is characterized by high specificity and high sensitivity, allowing precise discrimination of a single antigen and detection of very low concentrations of target molecules. It can also be converted into measurable signals such as color change, fluorescence, or enzymatic activity in laboratory assays. This principle represents the scientific foundation of all modern immunological techniques, including immunostaining and immunodetection, where it is used to detect proteins and biological molecules within cells and tissues. It is therefore a central tool in monitoring the quality, purity, and efficacy of biological drugs.

4. Immunostaining and Immunodetection Techniques Used in Biopharmaceutical Monitoring

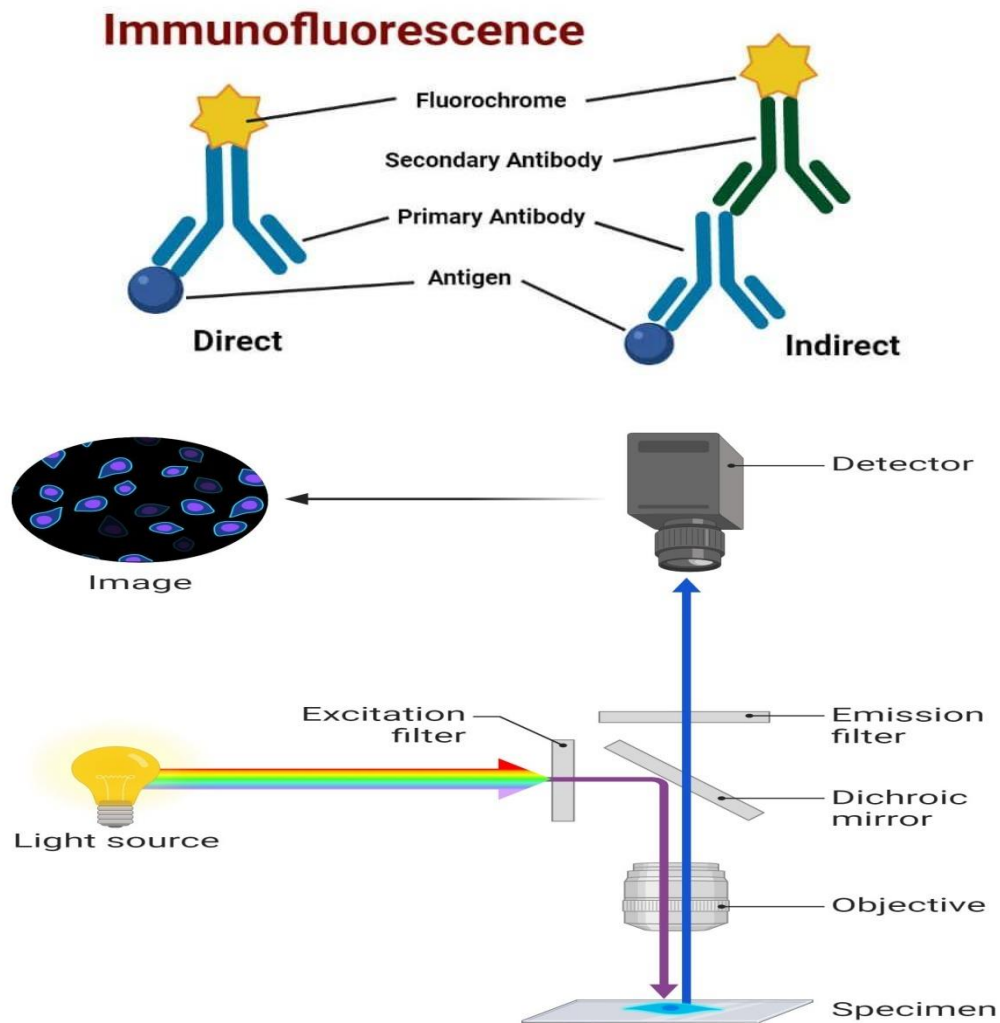
Immunological techniques used in the monitoring of biological drugs are classified into two main categories according to their purpose. These include immunostaining techniques, which focus on visualizing the location of molecules within cells or tissues, and immunodetection techniques, which are used to detect and analyze them qualitatively or quantitatively. This classification reflects differences in application and in the way results are obtained, allowing the appropriate technique to be selected according to the monitoring objective.

4.1. Immunostaining Techniques

Immunostaining techniques are used to visualize and localize biological molecules within cells or tissues based on antigen–antibody interactions, providing qualitative information about the spatial distribution of proteins. In biopharmaceutical monitoring, these techniques contribute to tracking the localization of the active substance or certain impurities within biological systems. This category includes several main techniques that differ according to the type of signal used and the detection method. The most important of these are:

4.1.1. Immunofluorescence

Immunofluorescence is one of the most important immunostaining techniques. It is based on the use of antibodies conjugated with fluorescent dyes (fluorophores) capable of emitting light when exposed to an appropriate excitation source. When the antibody binds to its target antigen, a fluorescent signal is produced that can be observed using a fluorescence microscope, allowing precise localization of biological molecules within cells or tissues. In biopharmaceutical manufacturing control, this technique is used to detect the distribution of therapeutic proteins within producing cells or to identify certain protein impurities. It also contributes to studying cellular interactions related to drug efficacy by tracking its binding to cellular targets, monitoring its internalization into cells, and observing changes in intracellular protein distribution. It can also be used to monitor activation or inhibition of cellular pathways by detecting the appearance or disappearance of specific proteins following drug exposure, which indicates modulation of a biological pathway. This technique is characterized by high sensitivity and the ability to provide precise spatial localization of molecules. However, it requires specialized equipment such as a fluorescence microscope, and the fluorescent signal may be affected by factors such as light intensity and temperature.



Immunofluorescence Microscopy:

- When an antibody, or the antiimmunoglobulin antibody used to detect the antibody is labeled with a fluorescent dye
- This method is used when looking at the subcellular location of a protein of interest

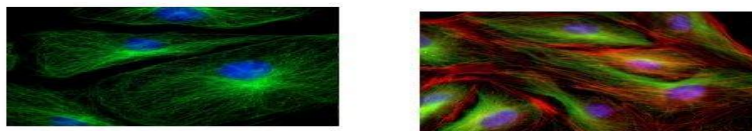


Figure 22: Cells or tissues after staining with fluorescent antibodies, where target proteins appear in bright fluorescent colors (green/red), illustrating the spatial distribution of the antigen within the cell or tissue.

4. 1. 2. Immunohistochemistry

Immunohistochemistry is a fundamental technique in immunostaining techniques, relying on antibodies conjugated to enzymes that catalyze a chemical reaction producing a visible color at the site of antigen localization within tissues. In this context, tissues refer to intact biological samples whose structural organization is preserved, allowing the study of molecular distribution within them. Upon antibody–antigen binding, the substrate is converted by the enzyme into a colored product observable under a light microscope, enabling precise localization of target molecules. In the field of biologic drug manufacturing monitoring, this technique is used to study the distribution of therapeutic proteins within tissues, and to detect impurities or structural changes that may occur during manufacturing or storage. These changes may appear as abnormal color signals or alterations in the pattern and intensity of distribution compared with the expected distribution of the active substance, which may indicate the presence of impurities such as undesired proteins or protein aggregates, or conformational changes affecting molecular structure and efficacy. It also contributes to evaluating drug–tissue biological interactions at the tissue level, particularly in preclinical studies. This technique is characterized by stable color signals and easy visualization compared with fluorescent techniques; however, it is less sensitive than immunofluorescence and requires longer preparation time.

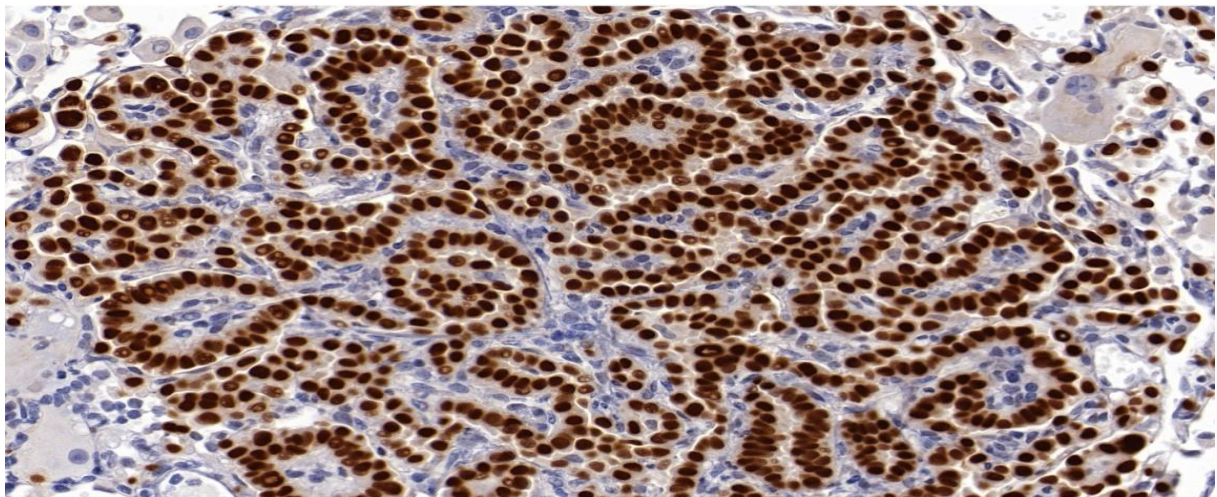


Figure 23:A tissue section stained with brown color (DAB), where the appearance of the color indicates the presence of the antigen within specific tissues, allowing the study of its distribution within the tissue architecture.

4. 2. Immunodetection Techniques

Immunodetection techniques are based on the principle of specific antigen–antibody interaction to detect the presence of target molecules or quantify them within biological samples. Unlike immunostaining techniques, which focus on localizing molecules within cells or tissues, these techniques are primarily intended for the qualitative or quantitative detection of proteins and biomolecules in solutions or complex samples, making them widely used in monitoring and evaluating the quality of biologic drugs during different production stages. These techniques are characterized by high sensitivity and precision, as they can detect very small amounts of therapeutic proteins or associated impurities. They are also widely used in monitoring active substance purity, verifying identity, and detecting contamination or deviations in the manufacturing process. This category includes several main techniques that differ in detection principles and the nature of the resulting signal, the most important of which are:

4. 2. 1. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is one of the most widely used immunodetection techniques in biologic drug manufacturing monitoring. It is based on the specific antigen–antibody interaction combined with an enzyme linked to one of the reaction components to produce a measurable signal, most often a color change whose intensity is measured using a spectrophotometer. It is used for the qualitative or quantitative detection of therapeutic proteins such as monoclonal antibodies or protein hormones, as well as for detecting host cell proteins or residual unwanted components resulting from the manufacturing process. It can also be used to detect certain microbial markers when the antibody is specifically designed for that purpose. The importance of ELISA lies in its high sensitivity, which allows detection of very low concentrations of target molecules, making it an essential tool for evaluating active substance purity and monitoring production batch quality. The main steps of the technique are as follows: one component of the reaction (antigen or antibody) is immobilized on a solid surface in a microplate, then the sample is added and the interaction occurs between components. After that, an enzyme-conjugated antibody is added, followed by the substrate, which is converted by the enzyme into a colored product. Between each step, a washing process is performed to remove unbound molecules or non-specific interactions, ensuring that

only the specific binding remains and improving accuracy. Finally, the color intensity is measured using a spectrophotometer, where the intensity is proportional to the amount of antigen present in the sample, allowing precise quantitative analysis.

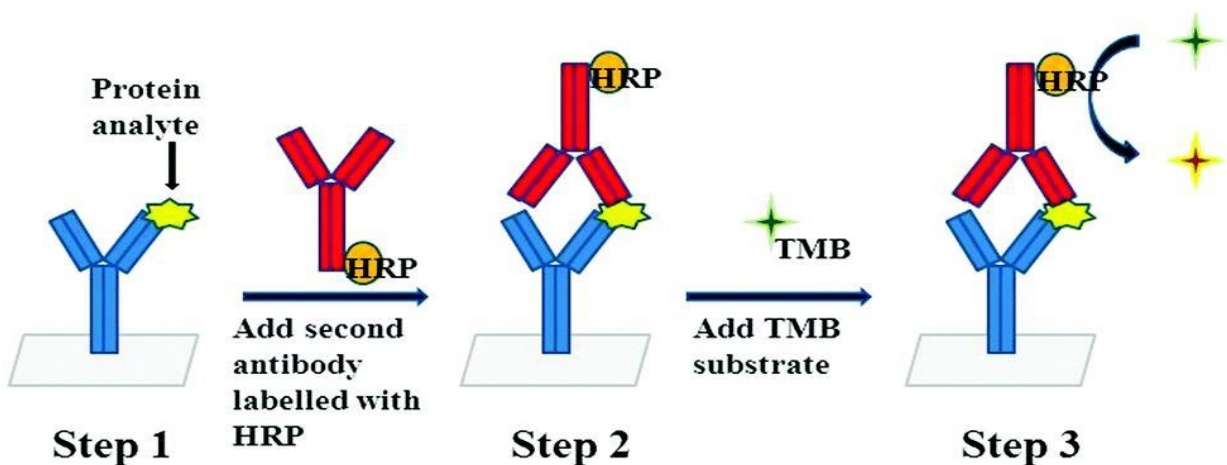
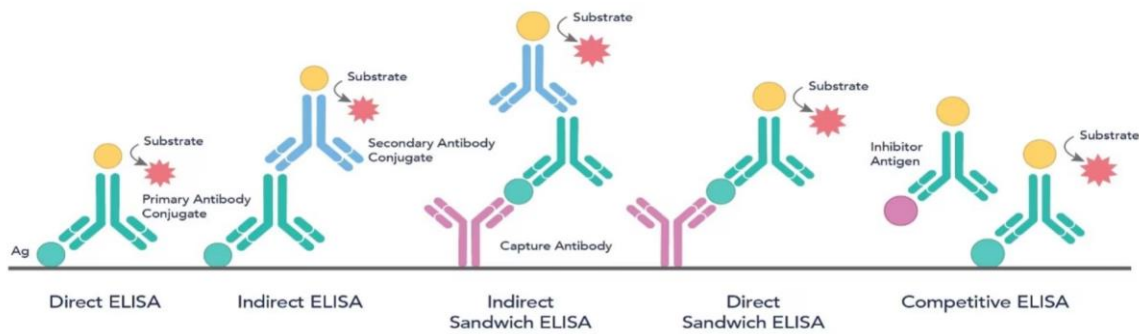


Figure 24 : A multi-well ELISA plate, where the color changes within the wells according to antigen concentration, and results are read using a spectrophotometer.

4. 2. 2. Western Blot

The term Western Blot is a conventional designation that emerged after the Southern Blot technique used for DNA detection, which was named after the scientist Edwin Southern, followed by the Northern Blot technique for RNA. The term “Western” does not refer to a geographical direction or a direct scientific meaning; rather, it is a linguistic extension used to designate the third technique developed for protein detection after separation and transfer onto a solid membrane through a blotting process. This technique is based on first separating proteins according to their molecular weight, then specifically detecting them using targeted antibodies, allowing precise identification of a specific protein within a complex protein mixture. The process begins with protein separation using electrophoresis, where proteins are separated according to size and charge within a gel matrix. The proteins are then transferred onto a solid membrane in a step known as blotting, where they are immobilized. Next, a primary specific antibody is added, which binds to the target protein, followed by a secondary antibody conjugated with an enzyme to generate a detectable signal, which may be colorimetric, chemiluminescent, or fluorescent depending on the detection system used. In the field of biologic drug production, this technique is used to confirm the identity of the therapeutic protein and verify its correct molecular weight. It is also used to detect protein impurities or unwanted proteins that may arise during the production process. It further assists in evaluating protein purity and structural integrity, making it an important tool for ensuring the quality of the final product and its compliance with specifications. Western Blot is characterized by high specificity and precise detection of a target protein within a complex mixture; however, it requires relatively longer processing time and multiple steps compared to techniques such as ELISA.

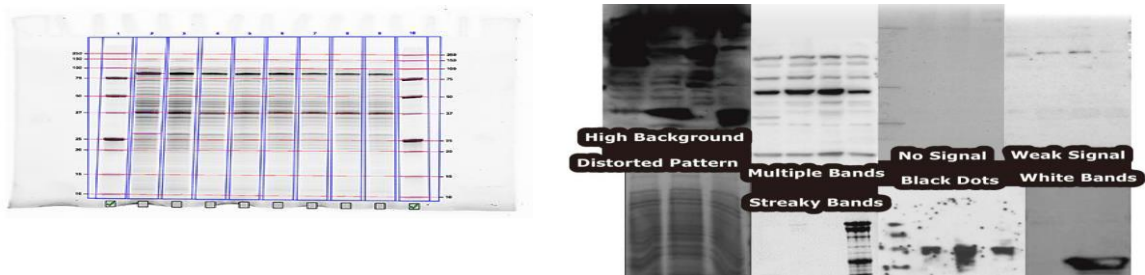
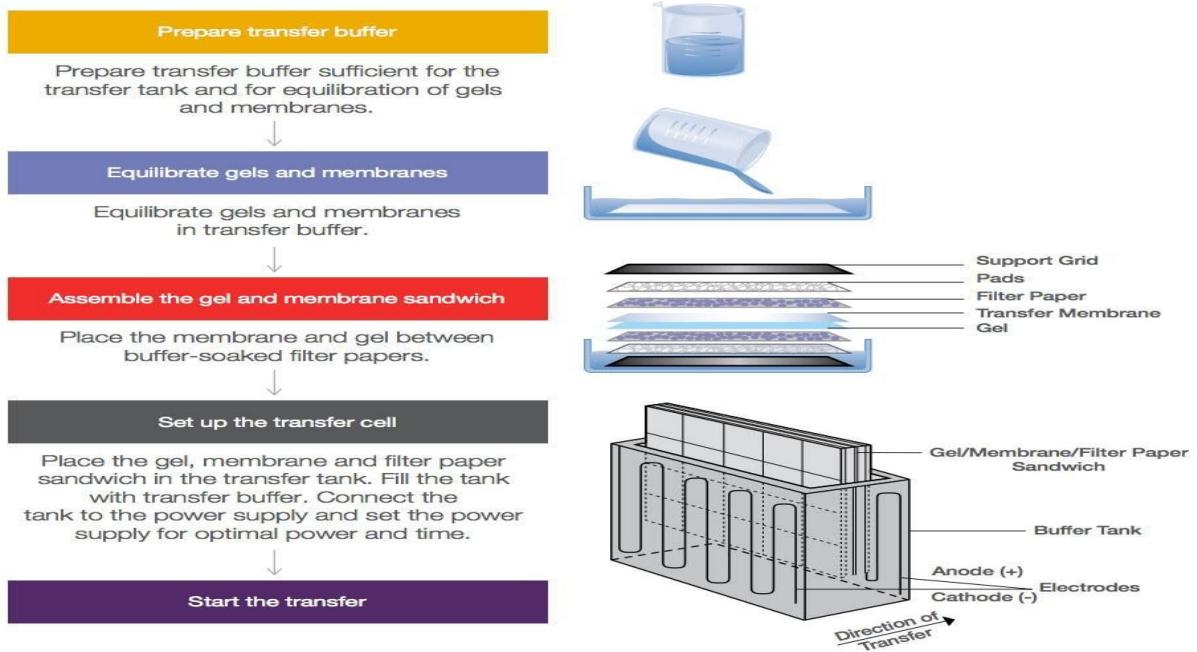
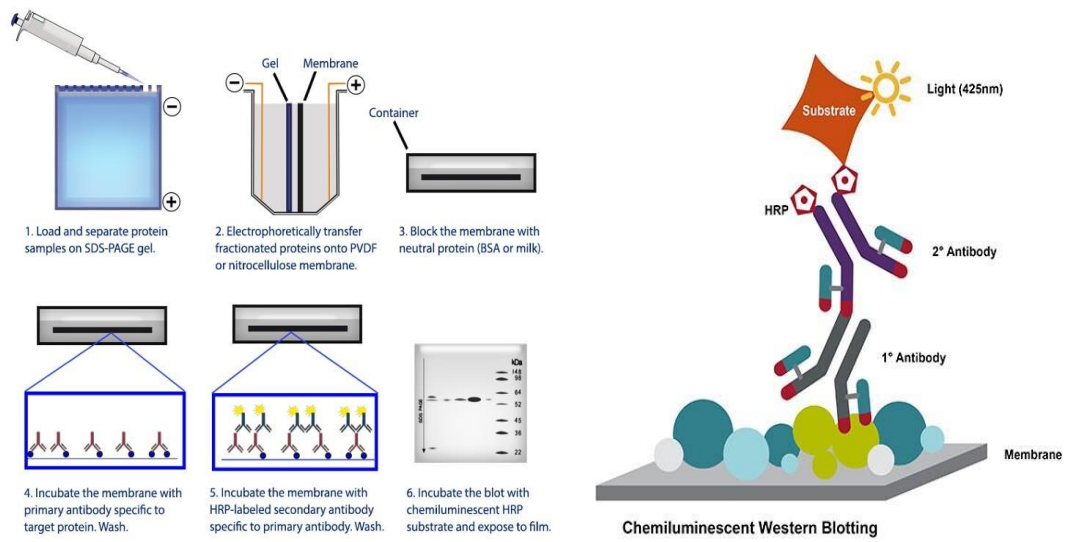
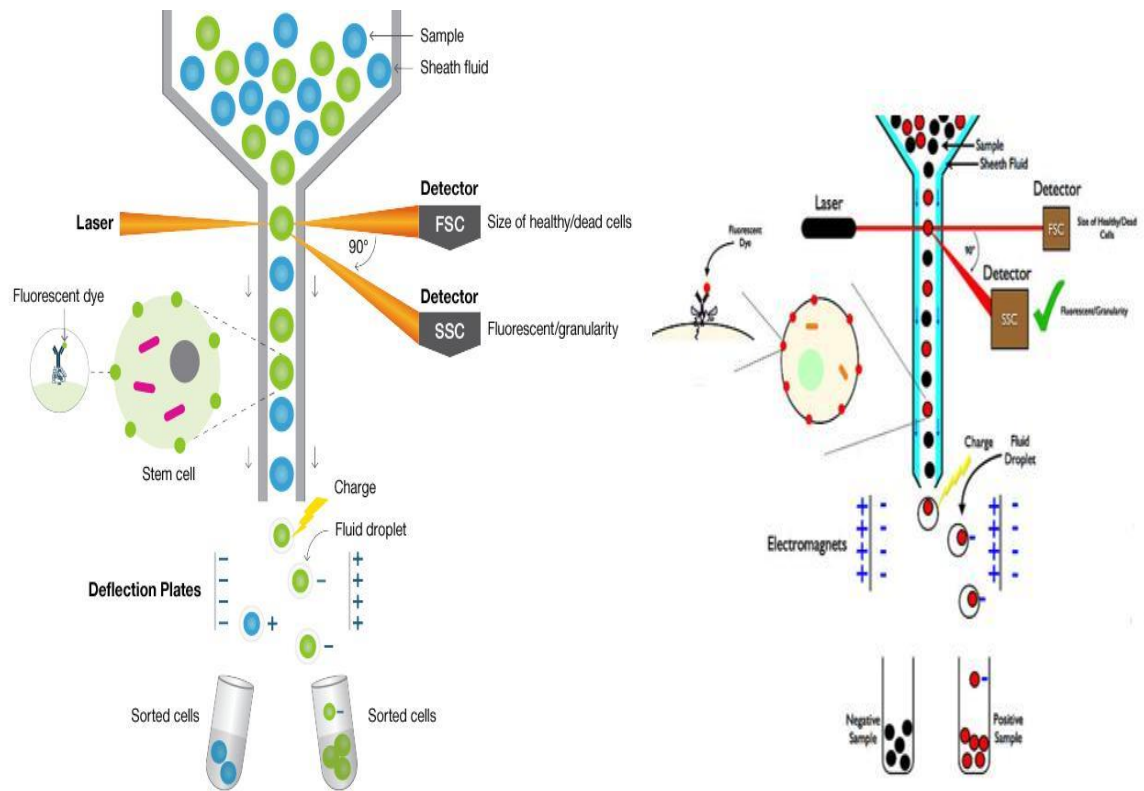


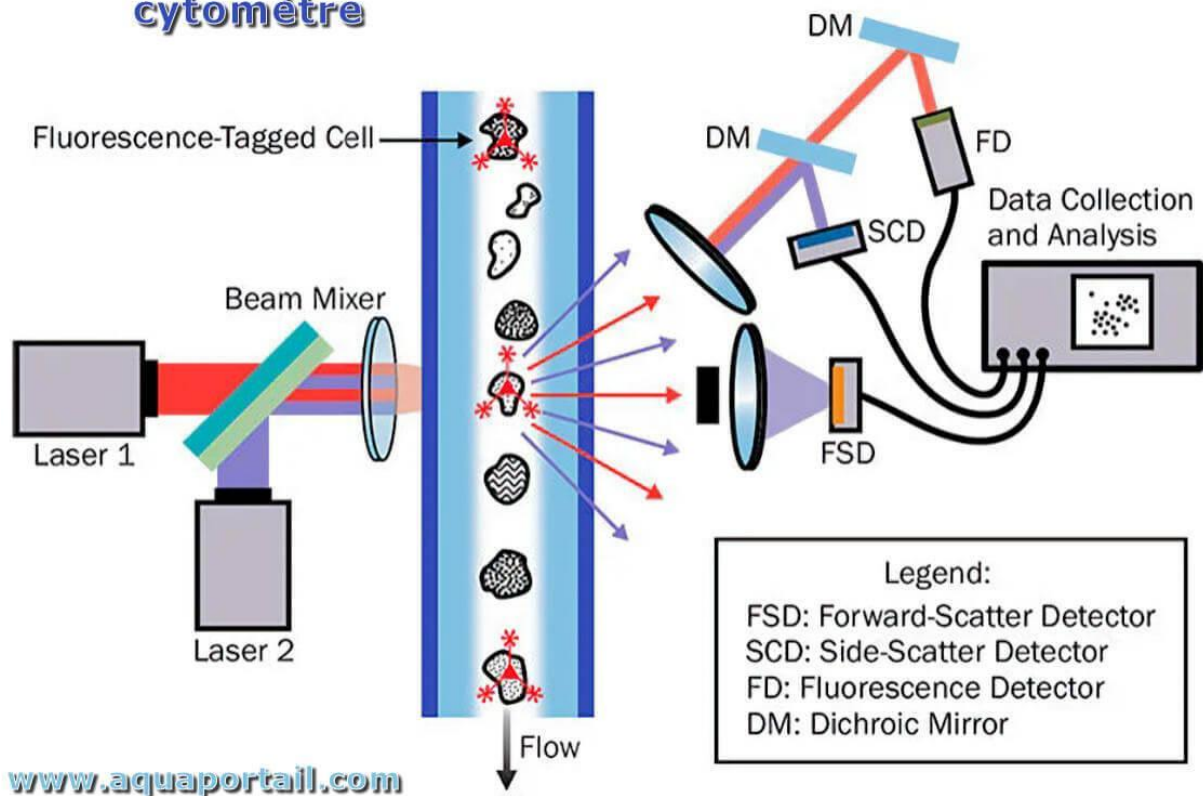
Figure 25 : Bands on a membrane representing proteins separated according to molecular weight.

4. 2. 3. Flow Cytometry-based Immunodetection

This technique is based on the analysis of individual cells as they pass through a narrow fluid stream in front of a laser beam, where optical signals generated from the interaction of fluorescently labeled antibodies with specific cellular components are measured. This principle allows the acquisition of precise information about each individual cell, such as its size, internal complexity, and the presence of specific proteins or receptors on its surface or inside the cell. In the field of biologic drug manufacturing monitoring, this technique is used to evaluate the effect of therapeutic proteins on target cells, and is also used to detect cellular impurities or changes in protein expression within production cells. In addition, it helps monitor the quality of cells used in bioproduction by analyzing their homogeneity and functional state, making it an important tool for ensuring the stability and efficacy of biologic products. This technique is characterized by its ability to rapidly analyze a large number of cells in a short time with high statistical accuracy; however, it requires advanced equipment and technical expertise for analysis and interpretation of results.



cytomètre



www.aquaportail.com

Figure 26: A flow cytometry instrument, where cells are analyzed one by one to reveal differences in size and protein expression.

5. Applications of Immunological Techniques in Biopharmaceuticals

Immunological techniques, whether immunostaining or immunodetection techniques, are essential tools in monitoring the manufacturing of biologic drugs. They are applied at different production stages to ensure the quality, efficacy, and safety of the final product. Their main applications include verifying the identity of the biologically active substance and confirming that it is the correct protein produced according to defined specifications through specific detection using targeted antibodies. They are also used to assess the purity of biologic products by detecting impurities such as host cell proteins, residual DNA, and undesired protein aggregates, as well as detecting potential microbial contamination during manufacturing or storage. They also contribute to monitoring the efficacy of biologic drugs by studying their binding to cellular receptors and distribution within cells or tissues, which improves understanding of their biological behavior *in vivo*. Another important application is in in-process control, where samples are analyzed at each production stage to ensure that no structural or compositional deviations occur, as well as in evaluating protein stability during storage and transport. Furthermore, these techniques are used in preclinical and clinical studies to assess biosafety and immune responses to the drug. Thus, immunological techniques represent a fundamental pillar in biopharmaceuticals, providing precise tools that combine qualitative and quantitative analysis and ensuring the development of safe, effective, and compliant therapeutic products.

6. Advantages and Limitations of Immunological Techniques

Immunological techniques in biopharmaceutical applications demonstrate significant importance in terms of analytical effectiveness and monitoring accuracy. However, their use is not without certain limitations that may affect their application under specific conditions. From this perspective, these techniques can be evaluated through two main aspects: their advantages and their limitations, in order to establish a balanced understanding of their role in biological drug quality control.

6.1 Advantages of Immunological Techniques

Immunological techniques used in biologic drug manufacturing monitoring are characterized by several properties that make them among the most important analytical tools

in this field. They are based on the specific antigen–antibody binding principle, which provides very high specificity, allowing discrimination between structurally similar molecules. They also exhibit high sensitivity, enabling the detection of very low concentrations of therapeutic proteins or impurities even at minimal levels. In addition, these techniques allow both qualitative and quantitative analyses, and they can be applied at different stages of production, making them flexible and effective tools for quality control and ensuring the safety of the final product.

6.2 Limitations of Immunological Techniques

Despite their significant advantages, these techniques present certain limitations that must be considered, as they may affect their use in some cases and should be carefully taken into account. One of the main limitations is the relatively high cost of some advanced techniques such as flow cytometry and immunofluorescence, in addition to the need for specialized laboratory equipment and highly skilled technical expertise for analysis and interpretation. Some techniques are also sensitive to environmental conditions such as temperature and pH, which may affect the accuracy of results. Furthermore, certain methods require longer processing time due to multiple experimental steps, such as ELISA and Western Blot, compared with faster analytical techniques. Nevertheless, despite these limitations, immunological techniques remain one of the essential pillars in biopharmaceuticals due to their high precision and their central role in ensuring product quality, safety, and therapeutic efficacy.

7. Conclusion

All analytical techniques play a vital and complementary role in ensuring the quality and safety of pharmaceutical and biologic products. Physicochemical analyses ensure formulation accuracy and dose consistency, while microbiological analyses confirm the absence of microbial contamination such as bacteria and fungi. Staining techniques and immunological assays elevate quality control to a more specialized and precise level. Immunological detection techniques rely on highly specific antigen–antibody interactions and are widely used in the pharmaceutical and biotechnological industries to detect host cell protein residues – HCPs resulting from the production of biologic drugs such as insulin and

monoclonal antibodies in living cells (bacterial or mammalian). After purification processes, it is essential to ensure their complete removal to avoid severe allergic reactions. They are also used to verify the identity and specificity of the active biological substance, ensuring conformity with the intended target and the absence of similar unwanted proteins. In addition, they allow precise detection of biological impurities as well as endotoxins and pyrogens produced by bacteria even after cell death, which may cause fever and serious complications upon injection. Moreover, they are used for accurate quantitative measurement of biologics such as sera, vaccines, and protein hormones, with ELISA being one of the most widely used techniques. Staining techniques are used as rapid visual and confirmatory tools under the microscope to identify the nature and type of microbial contaminants. Microbiological analysis does not only detect the presence of microorganisms; techniques such as Gram staining help determine whether bacteria are Gram-positive or Gram-negative, which contributes to identifying possible contamination sources such as hands, water, or air. Spore staining techniques are used to detect spore formation resistant to sterilization processes, which is an important indicator for evaluating the efficiency of aseptic production lines. They are also used to assess cell safety and product morphology in modern cell therapies through the differentiation of live and dead cells using dyes such as methylene blue or trypan blue. Thus, microbiological analysis determines the presence of microorganisms in general, staining techniques determine their identity and morphology, while immunological techniques detect the finest protein, biological, and toxic traces, ensuring the highest level of biosafety and patient safety. Immunological techniques, including immunostaining and immunodetection methods, play a central role in biologic drug manufacturing monitoring due to their high specificity and precise targeting of biomolecules. Their continuous development has improved evaluation and analysis processes across all production stages, from identifying the active substance to detecting impurities and assessing purity, efficacy, and stability of final products. Although highly precise and sensitive, these techniques remain associated with certain technical and cost-related limitations, requiring appropriate selection according to the intended analytical objective. Therefore, immunological techniques represent a fundamental scientific pillar in the development and monitoring of modern biologic drugs and directly contribute to improving therapeutic product quality and ensuring compliance with pharmaceutical standards.

*General
Conclusion*

General Conclusion

This course handout addresses the topic of monitoring and control of biopharmaceutical manufacturing as one of the fundamental aspects in ensuring pharmaceutical quality within modern pharmaceutical industries, particularly in the context of the rapid development of biotechnology and the increasing complexity of biological products compared with conventional drugs. This work provides a comprehensive framework that establishes a connection between the different stages of manufacturing and the strict quality control requirements, allowing an integrated understanding of the production process of biological drugs from development stages to the assurance of their safety and efficacy.

The first chapter focused on the general process of drug manufacturing by providing a systematic comparison between the production of conventional allopathic drugs and the manufacturing of biological drugs. This analysis highlighted the major differences between these two categories in terms of the source of the active substance, the degree of structural complexity, and the strict production conditions associated with the use of living biological systems. It was also demonstrated that biological drugs are characterized by their complex and highly specific molecules, such as proteins and monoclonal antibodies, making their manufacturing process more sensitive and requiring advanced and highly accurate technologies. In addition, the main therapeutic applications of biological drugs were reviewed, particularly their role in the treatment of chronic, immune-related, and oncological diseases, reflecting their increasing importance in modern medical practice.

The second chapter focused on quality control techniques in biopharmaceutical manufacturing (Quality Control in Biopharmaceuticals), where different levels of monitoring required to ensure the conformity of biological products with international quality standards were discussed. This monitoring includes physicochemical control techniques, which aim to evaluate the structural and functional characteristics of the product, as well as microbiological control techniques that ensure the absence of microbial contamination and guarantee the safety of biological preparations. Furthermore, toxicity assessment methods were addressed as essential steps for evaluating the safety profile of biopharmaceutical products before clinical use, according to strict regulatory requirements established by international pharmaceutical authorities.

The third chapter addressed immunostaining and immunodetection techniques as advanced analytical tools used for the identification and characterization of biological molecules within cells and tissues. These techniques are distinguished by their high specificity and accuracy in detecting target antigens, allowing the investigation of cellular distribution patterns of proteins and the understanding of molecular expression mechanisms within biological systems. Their contribution to research and development processes and quality control was also highlighted, particularly in the evaluation of biological product efficacy and the confirmation of their compliance with required specifications.

Overall, the integration of biopharmaceutical manufacturing processes, quality control strategies, and immunological analytical techniques represents a comprehensive scientific approach aimed at ensuring the production of safe, effective, and high-quality biological medicines. The continuous advancement of this field emphasizes the essential role of scientific research and technological innovation in improving manufacturing and monitoring approaches while meeting the requirements of modern pharmaceutical industries and international regulatory standards.

Bibliography

Bibliography

Abbas, A. K., Lichtman, A. H., & Pillai, S. (2021). *Cellular and molecular immunology* (10th ed.). Elsevier.

Ahuja, S., & Scypinski, S. (2022). *Handbook of modern pharmaceutical analysis* (2nd ed.). Academic Press.

Agalloco, J., & Akers, J. (2021). *Aseptic processing: A review of current industry practice* (4th ed.). CRC Press.

Akers, M. J. (2021). Protein formulation and stabilization in biopharmaceuticals. *Journal of Pharmaceutical Sciences*, 110(3), 1021–1035.

Alhazmi, H. A., & Albratty, M. (2023). Analytical techniques for characterization of monoclonal antibodies. *Pharmaceuticals*, 16(2), 291.

Almutairi, A. A., et al. (2020). The use of biologics in autoimmune disease management: A review of recent advances and challenges. *Journal of Population Therapeutics and Clinical Pharmacology*.

Allen, L. V. (2022). *Pharmaceutical dosage forms and drug delivery systems* (12th ed.). Wolters Kluwer.

Aulton, M. E., & Taylor, K. (2021). *Aulton's pharmaceuticals: The design and manufacture of medicines* (6th ed.). Elsevier.

Carpenter, J. F., & Manning, M. C. (2022). Rational design of stable protein formulations. *Nature Reviews Drug Discovery*, 21, 789–805.

Chen, X., Li, Y., Zhang, H., & Wang, J. (2022). Current trends in biopharmaceutical production in *Escherichia coli*. *Biotechnology Letters*, 44(8), 917–931.

Dahlgren, G., & Hausner, D. (2023). ICH Q13 and the future of continuous manufacturing. *Pharmaceutical Engineering*, 43(4), 24–31.

Denyer, S. P., Hodges, N. A., & Gorman, S. P. (2020). *Hugo and Russell's pharmaceutical microbiology* (9th ed.). Wiley-Blackwell.

European Medicines Agency (EMA). (2023). *Guidelines on quality, manufacturing, and non-clinical safety of biological products*. EMA.

Florence, A. T., & Siepmann, J. (2023). *Modern pharmaceuticals* (6th ed.). CRC Press.

Food and Drug Administration (FDA). (2022–2023). *Guidances for industry: Biopharmaceuticals, sterile products, and continuous manufacturing*. U.S. FDA.

Freeman, R., & Fan, J. (2022). Formulation development for biologics: Challenges and strategies. *Journal of Pharmaceutical Sciences*, 111(6), 1446–1459.

Bibliography

Gad, S. C. (2023). *Pharmaceutical manufacturing handbook: Production and processes* (3rd ed.). Wiley.

Goudar, C. T., Agrawal, G., & Rao, B. M. (2021). Cell culture bioprocessing: Mammalian platforms and bioreactor considerations. *Current Opinion in Chemical Engineering*, 34, 100714.

International Council for Harmonisation (ICH). (2023). *ICH Q6B, Q10, Q13 guidelines*. ICH.

ISO. (2015). *ISO 14644-1: Cleanrooms and associated controlled environments*. International Organization for Standardization.

Javanmard, S., & Yildirim, Y. (2023). Cell lines for monoclonal antibody production. *International Journal of Pharmaceutical Investigation*, 13(3), 381–392.

Joglekar, G. S., & Yadav, V. G. (2024). Process analytical technology in downstream processing. *International Journal of Pharmaceutics*, 661, 124412.

Jozala, A. F., & Mazzola, P. G. (2018). Downstream processing in biopharmaceuticals. *Journal of Chemical Technology & Biotechnology*, 93(7), 1753–1765.

Kumar, N., & Singh, R. (2023). Advances in mammalian expression systems. *Journal of Biotechnology*, 361, 72–85.

Li, F. (2018). Manufacturing of biopharmaceuticals. *mAbs*, 10(5), 607–620.

Li, D., et al. (2025). FDA-approved small molecule drugs: Advances and prospects. *Chinese Chemical Letters*.

Mahdi, G., et al. (2025). Biopharmaceutical analysis by HPLC. *Chonnam Medical Journal*, 61(1), 19–31.

Malakar, S., et al. (2024). Cancer treatment with biosimilar drugs. *Cancer Innovation*, 3(2), e115.

Martins, A. C., et al. (2025). FDA-approved monoclonal antibodies and proteins. *Biomedicines*, 13(8), 1962.

Murphy, J. (2023). Guidance on continuous manufacturing (ICH Q13). *Pharmaceutical Technology*.

Park, G., et al. (2025). Brief guide to immunostaining. *Molecular and Cellular Biology*, 48(1), 100157.

Piña, R., et al. (2022). Advances in immunofluorescence optimization. *International Journal of Molecular Sciences*, 23(3), 1426.

Rathore, A. S., & Kapoor, G. (2022). Biopharmaceutical manufacturing considerations. *Journal of Pharmaceutical Sciences*, 111(6), 2001–2015.

Bibliography

- Rosano, G. L., & Ceccarelli, E. A. (2014). Protein expression in *Escherichia coli*. *Microbial Cell Factories*, 13, 73.
- Rosenberg, J. N., & Clarke, C. (2020). Downstream processing strategies. *Biotechnology Advances*, 43, 107574.
- Sandle, T. (2023). *Pharmaceutical microbiology: QA and QC* (2nd ed.). Elsevier.
- Shukla, A. A., & Thömmes, J. (2021). Downstream processing of biopharmaceuticals. *Trends in Biotechnology*, 39(5), 432–444.
- Southey, M. W. Y., & Brunavs, M. (2023). Small molecule drug discovery. *Frontiers in Drug Discovery*.
- Szkodny, A. C., & Lee, K. H. (2022). Biopharmaceutical manufacturing: Historical perspectives. *Annual Review of Chemical and Biomolecular Engineering*, 13, 141–165.
- Tucker, G. T., & Harrison, D. J. (2021). Cold chain distribution for biopharmaceuticals. *Pharmaceutical Technology Europe*, 33(4), 22–31.
- United States Pharmacopeia (USP). (2024). *USP–NF general chapters and monographs*. USP Convention.
- Walsh, G. (2018). Biopharmaceutical benchmarks. *Nature Biotechnology*, 36, 1136–1145.
- Walsh, G. (2023). *Biopharmaceuticals: Biochemistry and biotechnology* (3rd ed.). Wiley.
- Wang, D., et al. (2023). Immunoassay technologies for infection detection. *Frontiers in Cellular and Infection Microbiology*, 12, 1040248.
- Wang, W., et al. (2022). Stability of protein pharmaceuticals. *Journal of Pharmaceutical Sciences*, 111(3), 681–702.
- World Health Organization (WHO). (2022). *Guidelines on biotherapeutic protein products and good manufacturing practices*. WHO.
- Zhang, L., et al. (2024). Small molecule anticancer drugs. *European Journal of Medicinal Chemistry*.