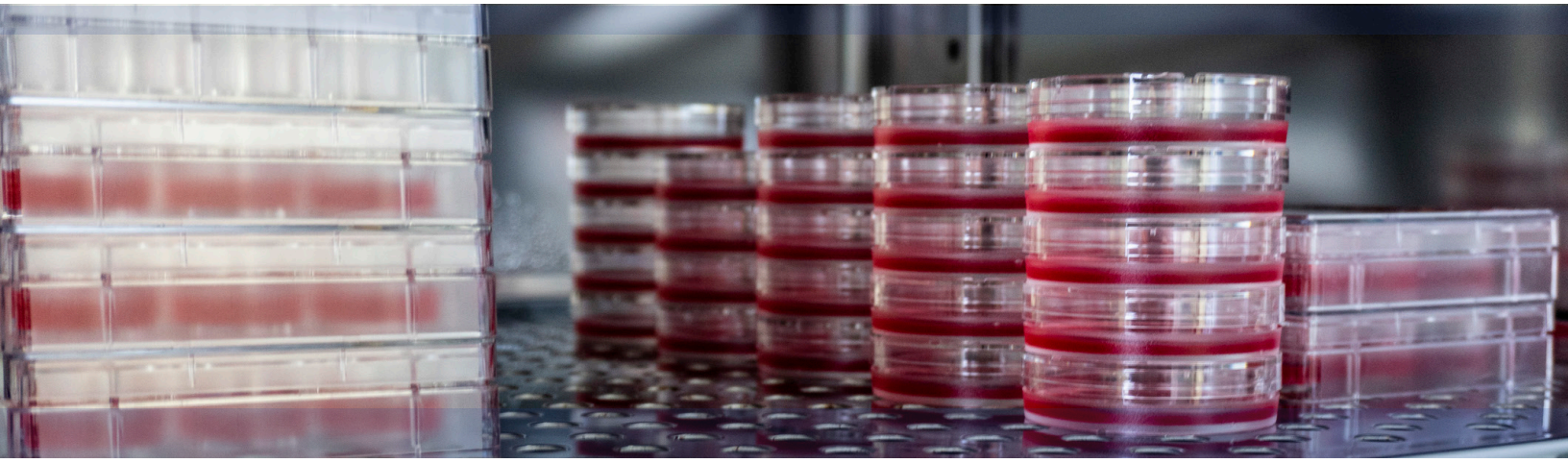
Maintaining Batch-to-Batch Consistency



Introduction

Biopharmaceutical production is a complex and multi-faceted process that encompasses inherent variability. The heterogenous nature of biopharmaceuticals makes them sensitive to subtle changes during manufacturing, processing and storage, that can impact their safety and efficacy in clinical use.[1,2,3] Understanding, defining and controlling such product variability is therefore a central challenge for all biopharmaceutical manufacturers.

Although it is difficult to assess the full impact of failure rates, it has been estimated that 7.2% of batches are lost every year.[4,5] The main source of error during biomanufacturing is batch contamination, which accounts for 2.3%. Figure 1 below highlights other reported sources of batch failure.[4,5]

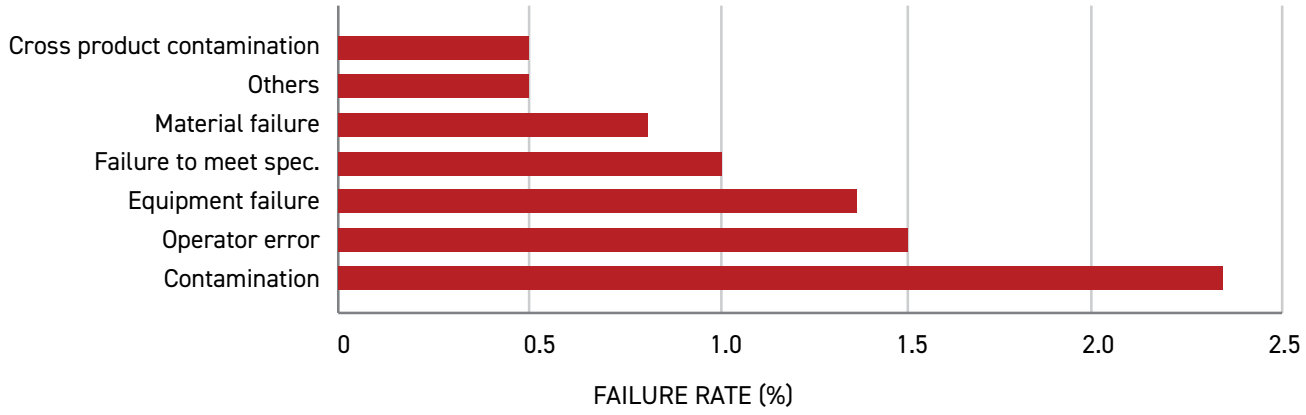


Figure 1. Reasons for batch failure below a size of 100L. Adapted from ([4,5]).

Critical quality attributes (CQA) relate to the physical, chemical, biological or microbiological properties and characteristics of biopharmaceuticals that impact their overall desired product quality. Changes to a biopharmaceutical's CQAs will ultimately affect its safety and efficacy in clinical use.[1,2,3] Identifying CQAs for a given biotherapeutic is the first, and arguably the most difficult, step in the development and production process.[6,7]

Managing the manufacturing process

Biopharmaceutical manufacturing can be broadly divided into four stages (Figure 2):[8,9]

1. Development: refers to the scale-up of processes to produce the quantities required for pre-clinical and clinical trials and manufacturing. Once the cell line is selected, genetic engineering is used to express the biomolecule of interest, resulting in a master cell bank that contains approximately one million recombinant cells – all expressing the target biomolecule. The master cell bank is stored at low temperature until the start of the next stage.

2. Upstream processing: the cell bank is used to produce large, stable, and controlled cell cultures. The cultures are maintained in bioreactors filled with growth media and are provided with the required nutrients and additives. Maintaining stable cell culture conditions is essential to reduce the variability of the biomolecule's CQAs. Typically, this stage ends with the harvest of the cell culture medium containing the target biomolecule.

3. Downstream processing: involves the extraction of the target protein from the harvested cells/culture supernatant. This stage relies on column chromatography and filtration to remove unwanted impurities (e.g., viruses, host cell proteins and DNA, aggregates, and endotoxins, etc.) to obtain a highly purified biomolecule. The product is then concentrated using ultrafiltration and diafiltration.

4. Formulation and fill processing: during this stage, the buffer conditions are optimized (e.g., pH, and ionic strength, etc.), and excipients and stabilizers are added to the purified biomolecule to obtain a stable pharmaceutical product. The final product is filled into vials, labeled, and packaged before final release.

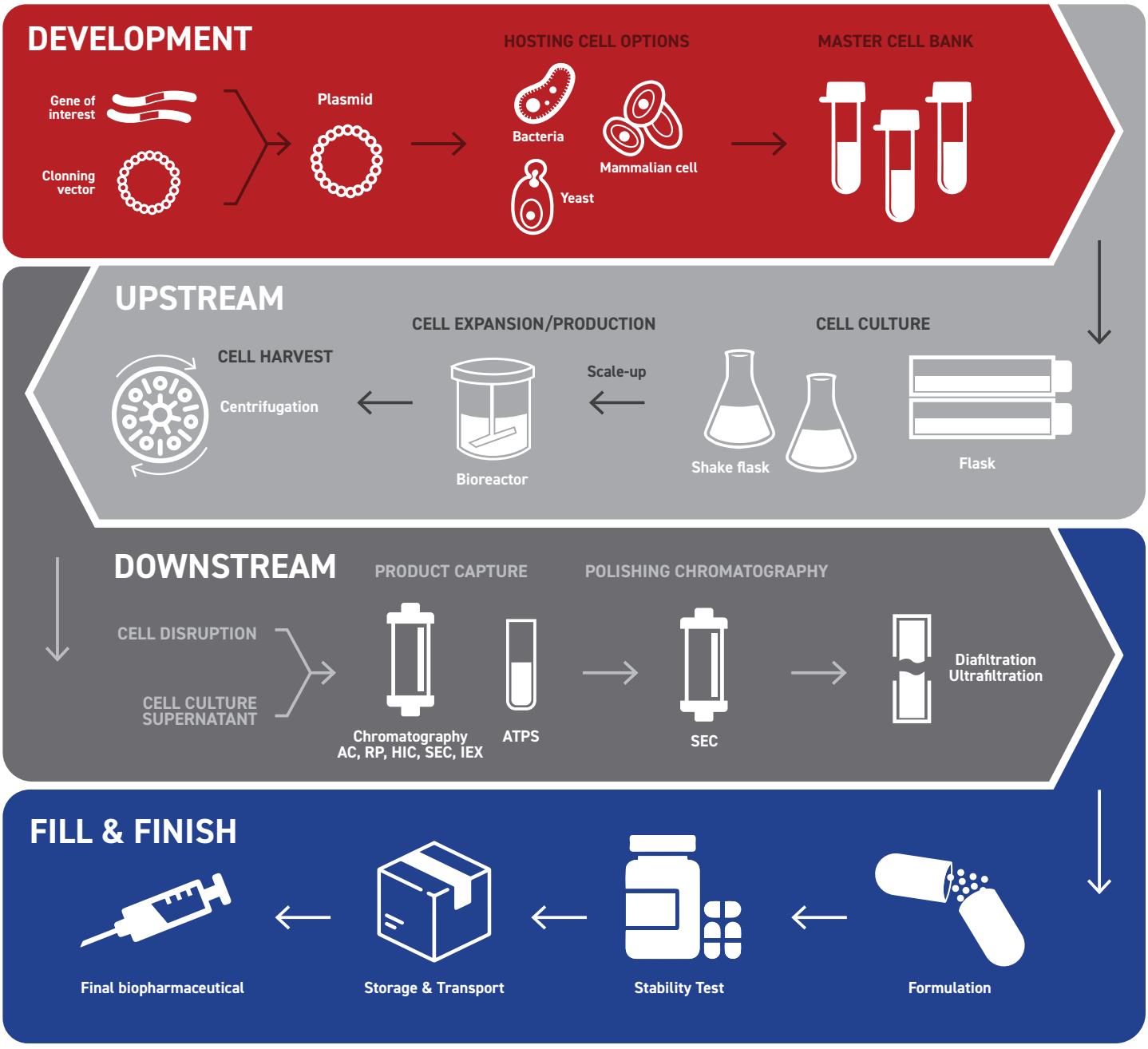


Figure 2. Outline of biopharmaceuticals manufacturing process.[10]

It is important to use high quality reagents and validated equipment throughout the entire manufacturing process. Additionally, key parameters (e.g., composition of the growth media, temperature, pH, and gases, etc.) should be continuously monitored as the slightest deviation from optimal conditions can affect the biomolecular properties and, subsequently, affect patient safety.[11]

What is batch-to-batch consistency?

Variability is inherent to the biopharmaceutical manufacturing process; therefore, it is essential to put control strategies in place to increase batch-to-batch consistency.

Common causes of batch variability include:[1,2,3,12]

1. Biological variation: Biomolecules are susceptible to post-translational modifications (PTM) which can change their physical/chemical properties, activity, localization, and/or stability. The type and number of PTMs that occur during the manufacturing process will depend on different factors including the type of cell line, cell culture conditions, purification strategy, formulation, and storage conditions. To minimize biological variation is important to ensure reproducibility of the conditions during all stages of the manufacturing process.

2. Analytical variation: Errors can occur while measuring critical variables (e.g., temperature, pH, and O_2 concentration, etc.) due to equipment miscalibration or malfunction. Thus, maintenance and calibration of all equipment are essential to assure a reliable production.

3. Reagent impurity: Consistent cell growth depends on the purity of elements incorporated into the growth medium. Impurities and/or contamination (e.g., bacteria, mycoplasma, and viruses) can lead to differential cell growth and decrease batch-to-batch consistency.

4. Changes to the manufacturing processes: Manufacturers will occasionally need to modify processes or testing methods to offset the impact of changes in regulatory requirements, equipment or suppliers that may negatively affect batch consistency. Comparability assessments must be performed to assess the quality attributes before and after changes to a manufacturing process to ensure that there is no adverse impact on the clinical performance of a product.

5. Human error: Operators can and do make mistakes than can affect batch-to-batch consistency. Examples include incorrect cleaning of the reactor between batches, the failure to follow standard operating procedures (SOP) or incorrect transcription of the production code into batch records.

All these sources of variability can affect the physical, chemical, biological, or microbiological properties or, quality attributes, of biomolecules. Although each biomolecule has many quality attributes, only a subset of these will have a direct impact on the efficacy and safety of the final product. CQAs are defined as the features that should be maintained within an appropriate limit, range, or distribution to ensure the final quality of the product is as desired.[9] Changes in a biopharmaceutical's CQAs can impact a product's toxicity, biological activity, affinity, and immunogenicity. Identifying CQAs as early as possible in the pre-clinical product development process, therefore, ensures better decision making at each step along the translation process and confidence that an observed effect is reproducible in the clinical phase.[13] Table 1 shows typical CQAs for a therapeutic antibody and their effects on efficacy and safety.

Table 1. Typical critical quality attributes of a therapeutic antibody (adapted from[9]).

	CQA	EFFICACY	SAFETY
Structure	High-order structure	Misfolding or truncation decrease efficacy	Misfolding can lead to anti-drug antibody (ADA) formation
	Aggregates	Variable impact on binding efficacy	Higher aggregates can lead to ADA formation
	Oxidation	Can negatively impact potency	
Content	Protein concentration	Can impact dose/potency	
Glysoylation profile	High mannose	More effective with higher mannose	Can elicit immunogenic response
	Non-glycosylated forms	Negative impact on efficacy	Can elicit immunogenic response
Biological activity	Binding to particular receptors	Can impact mechanism of action (effector function)	
Process impurities	Polysorbate		Can be toxic
	Host cell DNA		Can elicit immunogenic response

The ideal cell culture environment

From basic research to clinical applications, cell culture has become an essential technique used in biopharmaceutical development. It is important that the cell culture environment mimics the cell physiological conditions as closely as possible. Therefore, the success of this technique relies on the careful control of many parameters, such as temperature, pH and the choice of growth media. Figure 3 outlines the physical and chemical conditions required to encourage cell proliferation and survival *in vitro*.[14]

The consequences of deviating from the optimal culture conditions can range from alterations of the target protein CQAs to a complete failure of the cell culture. Therefore, it is crucial to maintain high standards of reproducibility and reliability during all cell culture procedures. In this context, international guidelines, such as Good Cell Culture Practice

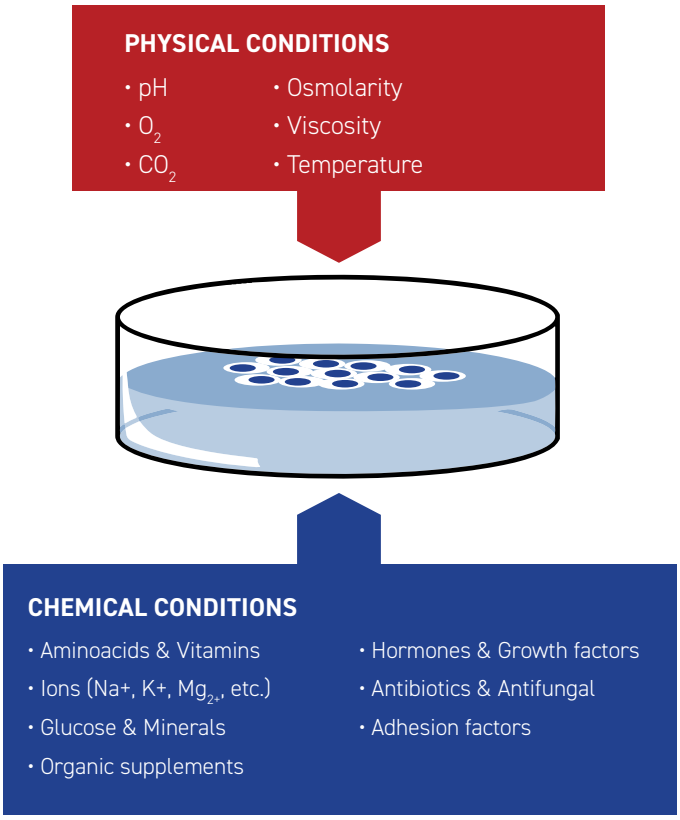


Figure 3. The parameters required for the successful growth and maintenance of a cell culture.

(GCCP)[15] and Good *In Vitro* Method Practices (GIVIMP),[16] help to maintain these standards reducing uncertainty in the development and application cell culture procedures and products. The recommendations outlined in these documents include: [14,16]

1. Education and training for *in vitro* techniques to help mitigate poor reproducibility caused by human error.

2. Adequate record and documentation of protocols (including materials, methodological details, conditions, equipment, etc.) to facilitate the replication of experimental conditions.

3. Implementation of quality assurance regimes to ensure consistency, traceability and reproducibility. All materials employed (i.e., cells, reagents, etc.) should be constantly monitored and stored under appropriate conditions to protect them from damage, infestation or contamination. Equipment and instruments must also be properly maintained and calibrated (e.g., temperature, and CO_2 control in incubators).

Taken together, these guidelines aim to increase reproducibility and encourage greater international harmonization, rationalization and standardization of laboratory practices.

The importance of reproducibility during bioprocessing

Despite the importance placed on reproducibility throughout the scientific process, more than 50% of preclinical research is deemed irreproducible. Such poor reproducibility rates, particularly at the cell culture stage, have a significant impact on subsequent upstream and downstream processing, leading to unsuccessful patient treatments and/or financial and administrative sanctions imposed by regulatory agencies. In addition to wasting time, money and resources, this can also jeopardize a company's reputation.[17] Recent initiatives targeted at improving the quality of *in vitro* research have identified critical aspects of *in vitro* cell culture routines and their influence on reproducibility (Figure 4).[18]

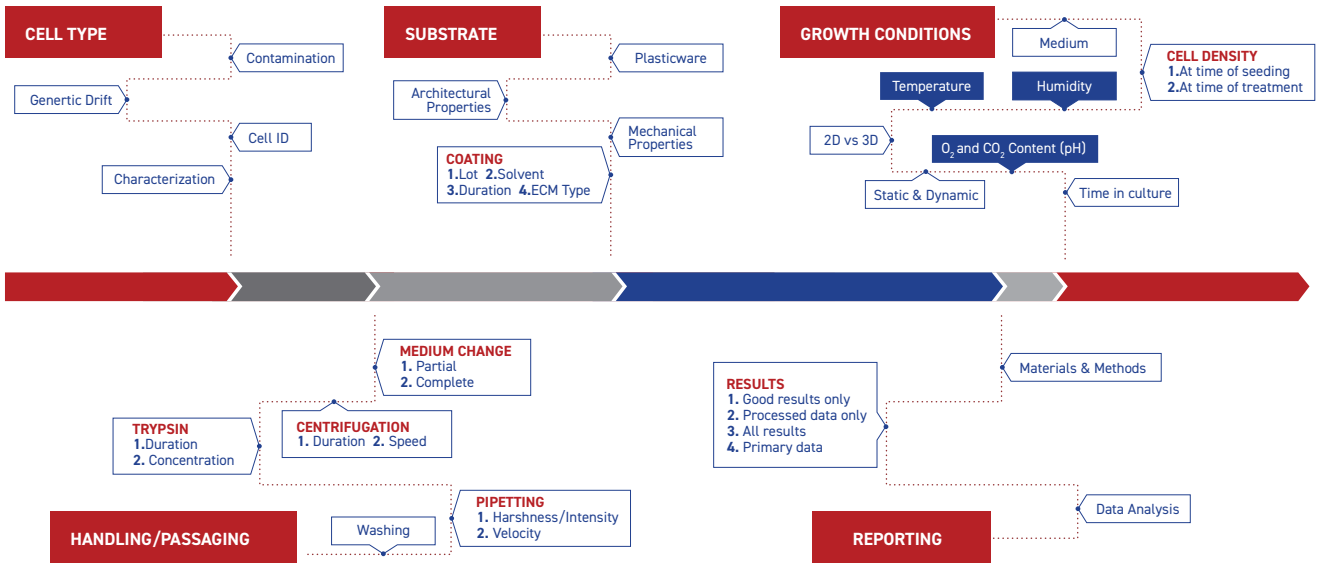


Figure 4. Summary of potential sources of variability influencing in vitro cell culture results (adapted from[18])

For example, it is widely known that most mammalian cells should be maintained at CO_2 levels between 5-7%, however, the control of O_2 levels is typically overlooked. While the physiological levels of O_2 in most tissues ranges between 2 and 11%, most cells are exposed to ambient levels between 18 and 21%, which often has a negative impact on culture phenotype.[19,20,21] To overcome this, multi-gas incubators can be used to maintain physiological levels for both CO_2 and O_2 and increase the reproducibility of cell cultures.

Humidity control is another variable that is crucial for cellular homeostasis. Small changes in humidity can significantly impact the rate of cell culture evaporation. When water evaporates from the cell culture media, the concentration of salts and minerals increases. This changes the osmolarity of the growing media, which can result in toxicity and cell death.[22] It is worth noting however, that high humidity also promotes the proliferation of bacteria and fungi, resulting in cell culture contamination. To minimize this risk and increase reproducibility, incubators should be regularly inspected and cleaned.

Using equipment to ensure reproducibility

All equipment and instrumentation should be optimized to ensure reproducibility and, ultimately, batch consistency. For example, frequently opening incubator doors can cause fluctuations in temperature, relative humidity and gas levels. To overcome this challenge, incubators can be equipped with direct Heat and Air Jacket systems, dual-infrared CO_2 sensors, and zirconia O_2 sensors to monitor these parameters more precisely and maintain uniformity. Contamination can be further minimized using InCu saFe® copper-enriched stainless steel alloy interior surfaces, $H20_2$ vapor decontamination systems and/or SafeCell UV light lamps. Table 3 below highlights the instrumentation required at each stage of the manufacturing process and the features that can be used to increase reproducibility.[23,24,25]

Table 3. Examples of the equipment required during biopharmaceutical manufacture.

DEVELOPMENT	EQUIPMENT	DETAILS
	Laminar flow hood (Class II and III)	Used to maintain an aseptic environment, avoid contamination of cell cultures, and protect the experimenter from potential hazardous materials.
	Incubators	Used to provide a stable and controlled environment for cell growth. CO_2 incubators control temperature and CO_2 concentration using IR sensors. Multi-gas incubators also allow for the control of O_2 concentration thanks to the addition of O_2 sensors. They can be equipped with H_2O_2 vapor decontamination system or SafeCell UV light lamps to reduce contamination.
	Inverted microscope	Used to monitor cell morphology and cell counting and to identify contaminants.
	Pharmaceutical refrigerators	Storing reagents and media between 2 and 8°C, helps to prevent sample loss. Refrigerators can be equipped with various securities and precise temperature control.
	Freezers	Most cell culture reagents can be stored between -5 and -30°C. Other material, such as DNA, RNA, or proteins require storage in an ultra-low temperature (ULT) freezer (-80°C) which offer safe short-term or long-term frozen storage of temperature-sensitive biological samples.
	Liquid nitrogen storage or Cryogenic freezer	Used for long-term storage of master cell banks at -150°C. Cryogenic freezers reduce the chances of cross-contamination and sample loss thanks to innovative features that enhance sample security (e.g., ID control for users, temperature monitoring, and dual cooling systems). Additionally, the use of vacuum insulation panels provides 30% more storage capacity and increases energy efficiency and cooling performance.
UPSTREAM	Bioreactor System	Used to grow high amounts of cells under highly controlled conditions. Bioreactor systems are large containers equipped with an agitator system (to maintain a homogenous environment) and different sensors (to control temperature, pH, and O_2 concentration, etc.).
	Harvest Systems	Large centrifugation systems are used to separate the cells from the media containing the target biomolecule.
	Clarification Systems	Used to remove debris from the harvested cells. The systems use various, sophisticated filters to remove particles of different sizes.
DOWNSTREAM	Filtration systems	This includes diafiltration (DF), ultrafiltration (UF), and microfiltration (MF) systems that remove unwanted impurities and concentrate the product.
	Chromatography systems	Used for purification and quality control. The systems include different types of chromatography columns that separate large volumes of material.
FILL & FINISH	Sterile filling systems	Used to fill the product under aseptic conditions into its final container (vials, syringes, and capsules, etc.) for distribution.

Conclusion

The complexity and inherently variable nature of biopharmaceutical production can significantly impact reproducibility and therefore batch consistency. In addition to the obvious impact on patient treatment and safety, batch variability can pose significant economic consequences for manufacturers; regulatory agencies can impose fines, penalties and bans due to non-compliance. Various control strategies must therefore be implemented throughout the product life cycle to ensure consistent clinical performance over time. The comprehensive characterization of CQAs (in line with international guidelines) and the selection of high-quality equipment are at the core of these strategies.

Since the approval of the first biopharmaceutical in 1982, only 0.01 % of the biopharmaceuticals introduced to the market experienced significantly changes in their clinical performance over time.[1] Such a low percentage proves that implementing modern analytics and manufacturing quality systems help to ensure reproducibility and therefore batch consistency. By implementing all these control strategies, patients can expect the same safety and efficacy from their biopharmaceutical products irrespective of batch or production history.

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