A Comprehensive Guide to Finding the Right Cell Culture Media for Your Bioprocesses

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Abstract

Cell culture media is a central component of an upstream bioprocess and a primary driver of cell viability and performance. Selecting and optimizing the media formulation and feed strategy in the process development phase is the key to a high productivity outcome in any bioprocess.

There is a vast range of off-the-shelf and custom media services to choose from, and getting the decision right is vital to establishing an efficient and reproducible manufacturing process. Cell type, application, and production goals drive the decision-making process, but their complex web of interactions can create media development challenges.

In this eBook, we provide an overview of cell culture media in bioprocessing and how strategies have evolved. We then discuss challenges and opportunities when developing a media approach during the production of biologics. Finally, we consider the different hurdles faced when selecting cell culture media during development and commercial manufacturing before revealing the benefits of partnering with a provider with expertise throughout the media pipeline.

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An Introduction to Cell Culture

Cell culture underpins the manufacture of essentially all protein- and cell-based biotherapeutics. Choosing the cell line and culture conditions for your application sparks the beginning of a journey towards developing a robust, productive bioprocess. The success of your manufacturing process and the quality of your final product is highly dependent upon the decisions made during upstream development, as these shape the entirety of your upstream process steps, directly impacting the performance of all downstream steps.

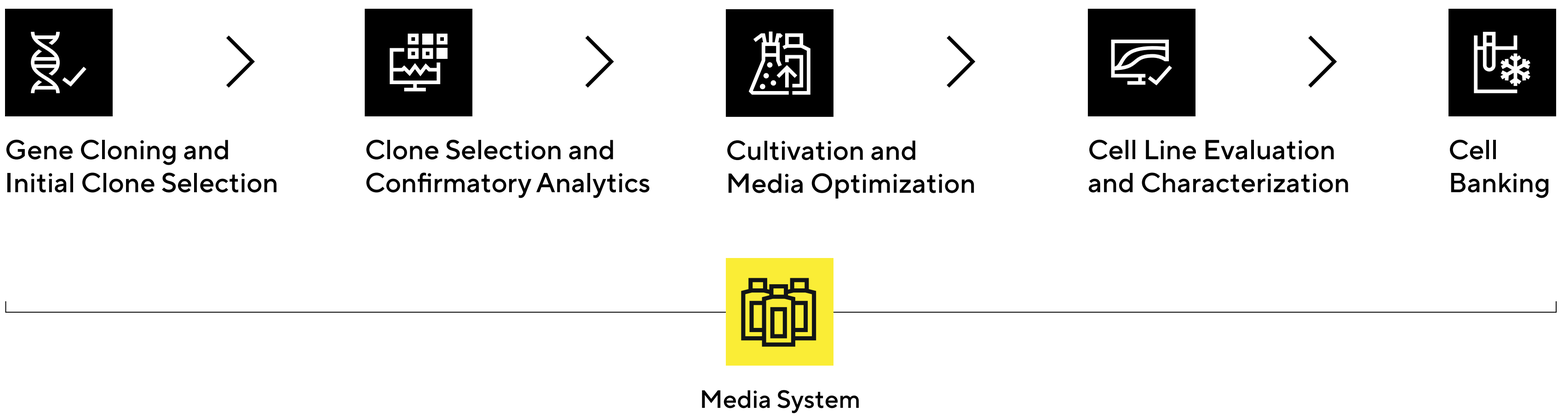
In establishing a protein or viral production process, getting cell line development right is crucial to maximizing productivity during cell culture steps. Cell line development is a multifaceted process: clone selection, media development, critical quality attribute (CQA) testing, and process parameter optimization are all essential components (Figure 1). Both the individual characteristics of the selected clone and the set-up of the process parameters strongly determine the behavior of the cell population and the production of the biologic, which can make designing an optimal upstream process a significant challenge.

For cell-based products such as CAR-T and stem cell therapy, the cell population is the final product. Therefore, the culture conditions are critical to the safety and potency of the therapeutic. Each modality presents different cell culture challenges, so it is vital to identify media and growth factor combinations that optimize the expansion and, if applicable, differentiation of the target cell type.

As part of process development, it is crucial to establish appropriate conditions for the cells to grow in vitro, as efficient growth conditions affect cell viability, proliferation, and production yields, ultimately dictating process performance. Monitoring and control of process variables such as pH, dissolved oxygen, dissolved carbon dioxide, and temperature are required to establish and maintain an optimal environment. Central to the cultivation of host cells and the production of biotherapeutics is the formulation of the growth medium, which has the utmost influence on optimal productivity.

Here, we discuss media attributes that favor high-performing and consistent cell cultures before delving into key considerations for developing media formulations, feeding strategy, and robust culture conditions as part of your upstream development process. Finally, we explain why accessing support from a provider with a vast product and service portfolio within the cell line development pipeline is crucial to getting the most out of your media strategy.

Figure 1: *A Typical CLD Process for Protein and Viral Vector Production*



An Overview of Cell Culture Media

The cell culture medium is a growth matrix containing all the factors required to recapitulate an optimal growth environment in which the cells can thrive. Media can maintain consistent pH and osmolarity conditions and provide nutrients to support cell proliferation.

The composition of the media strongly affects cell behavior and varies across cell lines, modalities, and process steps. For instance, the requirements of E. coli differ significantly from the requirements of human immune cells, and the needs of cells during transfection are different from when high yields of protein are required. Media that promotes rapid cellular growth does not necessarily also support high productivity. Therefore, it is crucial that biopharmaceutical developers optimize their media strategy as early as possible to maximize cellular performance and reduce the chance of failure.

”Selecting and optimizing the media formulation and feeding strategy in the process development phase is key to maximizing cellular performance and preventing costly failure.”

Mayank Gupta, M.Tech
Product Specialist
Cell Culture Media, Sartorius

The Evolution of Cell Culture Media

Initial biotherapeutic research and production were performed in simple host cells, such as bacteria and yeast. Improvements to our understanding of biological systems and technology allowed scientists to work with increasingly diverse and complex cell types that require specific media for their successful cultivation.

Bacterial expression systems are ideal for producing large volumes of expression plasmids and simple proteins. They are easy and relatively inexpensive to culture, and they can grow quickly to achieve high cell densities. However, bacteria lack the cellular machinery required to manufacture complex proteins and catalyze post-translational modifications, which may be required for a protein to fulfill its full therapeutic potential. Microbial nutritional requirements are mostly crude, and they can be grown in a simple broth without the need for additives such as hormones.

Expression systems have evolved over the years. Now, biotherapeutic manufacturers can take advantage of eukaryotic systems, which can support post-translational modifications and the production of more complex biomolecules, giving them broader utility. This increased complexity typically requires a more sophisticated medium to support cell proliferation and high-yield protein production.

Mammalian cells have particular value in the production of biotherapies, as the cellular machinery present in the cells can recapitulate the often vast array of post-translational modifications and complex protein folding and export required for the creation of therapeutic biologics. This is essential for creating recombinant proteins with all the CQAs necessary to provide effective therapy. In cell-based therapies, therapeutic cells are often derived from the patient; the media needs to harness biological properties that avoid complications like graft-versus-host disease (GvHD)[1] and promote rapid expansion to facilitate quick delivery back to the patient.

Classical vs. Modern Media

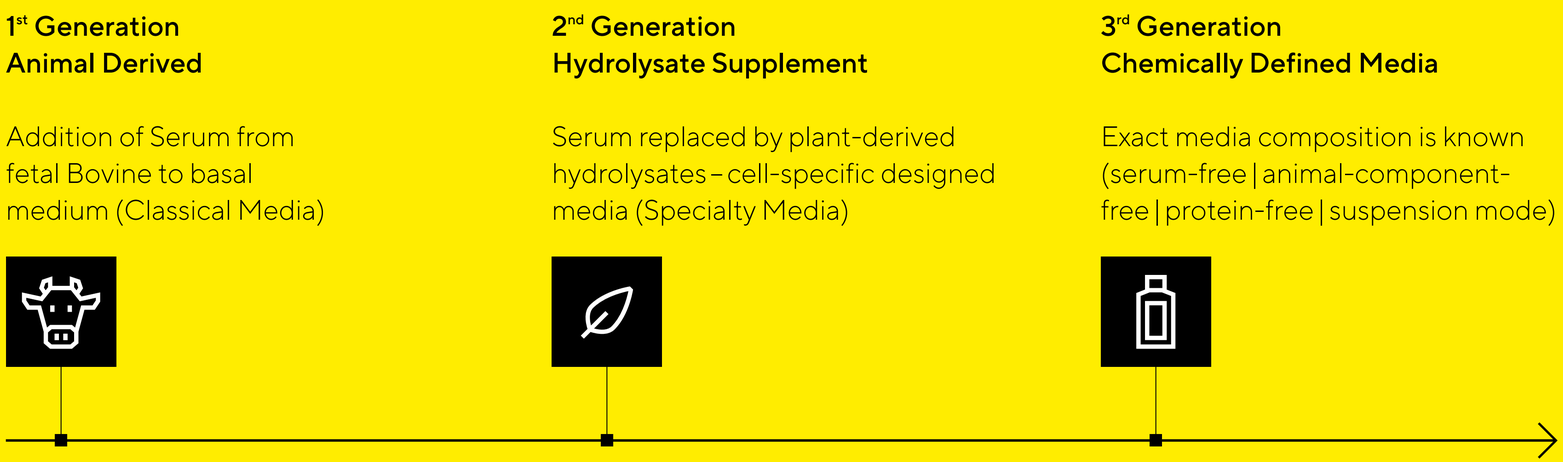
Traditionally, classical media involved the use of animal-derived serum, which contains growth factors and other proteins that support cell proliferation and survival. This system is still commonly used. However, as processes developed, manufacturers desired greater control to reduce variability and accelerate toward clinical applications, where reproducibility and safety are the top priority. As a result, cell culture media initially evolved to replace serum with hydrolysates, and now fully chemically defined media is becoming the norm in clinical bioprocesses (Figure 2).

The development of chemically defined serum-free media requires a complete understanding of each component and its role in cell metabolism. This includes energy sources, amino acids, lipids, vitamins, trace elements, protectants (particularly important for shear-sensitive cells), inorganic salts, nucleic acid substrates, and their components such as small molecule additives[1].

While most cells have been grown in classical media for decades, emerging media formulations are beginning to be highly tailored to the unique biology of each cell type and application. A variety of chemically-defined media have now been optimized to support the culture of different cell lines. The primary determining factor of media composition is the host cell line and the organism from which it is derived, which itself is determined by the biomolecule.

It is important to consider not just the formulation of the media but also the overall preparation, feed strategy, and storage system and how it fits into the overall upstream development and eventual manufacturing process. As well as the cell type, modality, and process step, these factors will be strongly influenced by the production stage (process development vs. commercial manufacturing).

Figure 2: *The Evolution of Cell Culture Media*



Considerations By Product

Recombinant Protein Production

When manufacturing recombinant proteins, the goal is typically to produce high yields of a good quality product. Therapeutic proteins are typically cultured in mammalian cells, and the choice of expression system is primarily determined by its ability to deliver high productivity while maintaining CQAs. A secondary feature might be the preference of the biotherapeutic developers, who may have had favorable experiences with a particular platform in the past.

The host cell line of choice to achieve high yields of a good quality product are Chinese hamster ovary (CHO) cells. CHO cells have historically been a workhorse in the industry: 70% of commercial recombinant proteins are produced in CHO cells[2]. There are several different CHO cell lines with distinct features and selection capabilities that are likely to require optimization of culture conditions. For example, CHO cells lacking the dihydrofolate reductase enzyme (DHFR) are among the most widely used CHO cells for the commercial production of biopharmaceuticals. This cell line – CHO DG44 – thrives in serum-free, chemically defined media and suspension growth. It is metabolically optimized for stable growth over long periods, making it ideal for meeting the requirements for large-scale protein expression and genetic stability.

CHO cells grow quickly and are typically cultivated at very high cell densities. In fed-batch production, they are typically maintained at around 20 – 30 million cells/mL. In perfusion culture, densities can reach 100 million cells/mL. Thus, they allow users to maximize the titer of their biologic while maintaining the CQAs.

Significant Media Demands of High-Density Cultures
High-density cell cultures allow users to maximize the titer of their biologic. However, sustaining high cell densities and maximizing productivity requires significant media development activities; sometimes, careful optimization of a single component can pay dividends in the future.

Dense cell populations will experience nutrient depletion quickly, which might require modification to the feed strategy or increased nutrients in the basal media. Additionally, the nutrients needed to support high cell density cultures might compete with those required for protein production. Therefore, it is vital to carefully determine the maximum cell densities a given medium can sustain for a required level of productivity.

Finally, with more cells, there will always be more host cell components in the spent media, creating downstream processing challenges. Choosing a protein-free media can help ease the challenges associated with the purification of your protein in a high-density harvest preparation.

Effect of Cell Culture Process Variables and Media on Glycosylation

Low glucose | glutamine concentration
- Reduces glycosylation and sialylation

Dissolved oxygen (DO)
- Cell line- and protein-specific effects

Bioreactor pH
- Galactosylation, sialylation and microheterogeneity affected

Manganese (Mn)
- Modulates the glycosylation profile
- Lack of Mn can inhibit O-linked glycosylation

pCO_2
- Decreases polysialylation
- Decreases N-Glycolylneuraminic acid (NGNA)

Ammonia
- High concentrations prevent terminal glycosylation

Sodium butyrate
- Protein-specific changes

Dimethylsulfoxide (DMSO)
- Decreases sialylation

Glycerol
- Enhances sialylation

Nucleotide-sugar content
- N-Acetylmannosamine supplementation enhances sialylation profiles

Glycine betaine
- Inhibits polysialylation at lower osmolalities <435 mmHg
- Protects polysialylation at higher osmolalities

Culture system?
- Stirred tank system decreases in sialylation

Figure 3: *Adapted from Hossler et al.*[4]

Tricky CQAs

The composition of the cell culture media significantly affects the physicochemical characteristics (e.g., glycosylation, sialyation, charge variants, and aggregation) of recombinant proteins such as monoclonal antibodies.

The glycosylation profile is an important CQA as it has a significant impact on the efficacy, safety, and half-life of the therapeutic. Various factors influence glycosylation profile (including clonal differences and cell culture conditions), but one of the simplest ways to modulate glycosylation patterns is by adjusting the levels of glucose and other carbon sources present in the media[2] (Figure 3). Thus, optimizing the balance of components in the media is critical to achieving a product with the desired characteristics. This is of particular interest in the creation of biosimilars, where the properties of the reference product must be faithfully recapitulated.

When tuning glycosylation to the required profile, traditional optimization methods such as titrating each component using a "one-factor-at-a-time" approach are valuable but labor-intensive and time-consuming. Screening these components across concentration ranges helps determine which elements strongly affect productivity and quality. Design-of-experiments (DoE) methods can streamline the experimental examination of these factors. During these optimization activities, changes in the osmolality of the medium and generation of metabolic wasteproducts should be carefully monitored.

Viral Vector Production

Various cell lines can be used for the production of viral vectors and vaccines, including HEK293, Vero, MDCK, and BHK-21. As previously indicated, the primary determinant of media composition will be the cell line, so media composition for viral vector processes is different than during the production of protein-based therapeutics.

Additionally, viral vector cell culture processes tend to operate at a lower density. In a batch operation, transfection | infection is typically performed at a cell density of around 1–2 million cells/mL. After five days, cultures reach a density of about 5–6 million cells/mL. Thus, the nutrient demands in these cultures will differ from the high-density CHO cultures used in protein production.

The infection should be performed in fresh medium with a subsequent medium exchange to maintain the cell culture at 2 million cells/mL cell densities while also improving productivity. Nutrients in the media should be sufficient to sustain the required cell density and a high virus replication rate. The choice of media impacts the maximum cell density before and during the infection phase while also influencing cell-specific productivity.

Fragility
Viral particles are typically much larger and often more fragile than proteins and are likely to be more sensitive to perturbations in pH and salt concentration. Thus, media may need to be carefully designed and its consumption tightly controlled to ensure deviations are kept to a minimum, and culture conditions may need to be kept gentle to prevent loss of yield or infectivity.

Diversity
Finally, because CHO-mediated recombinant protein production is well established, it has taken on more of a 'platform' shape. The industry is still a long way away from this for viruses, which are highly diverse in nature. For instance, even within one virus type (AAVs), over 100 serotypes have been identified[5], and each can behave very differently in culture. Multiple media should be tested to find the most suitable formula for each cell line and viral vector.

Physical characteristics are also highly variable between viral vectors, and their degree of accumulation inside cells versus media can differ significantly. This functional diversity creates a barrier to developing a platform process with high yield outcomes and reliable downstream recovery.

Cell-Based Therapy Production

Cell-based therapies typically involve modifying and culturing a patient's own cells or a donor's cells to create a target therapeutic. A new wave of approvals led by CAR-T therapies has drawn increased interest in using a variety of cell types (immune cells, iPSCs, MSCs) to treat diseases. As cells are the final product, the media and culture conditions are critical to the safety and potency of the therapeutic.

Additionally, since each modality presents different cell culture challenges, finding the media and growth factor combinations that support the robust growth of the target cell type is critical.

Persistence
Producing the target cell of interest—including subtype proportion (e.g., naïve T cells)–is tied to the media formula and growth factors used. To increase the effectiveness of cell-based therapies, they must be able to persist *in vivo*. Media components have been shown to impact the persistence of CAR-T therapies[6]. Therefore, a variety of media formulations must be screened to determine which elements are necessary to provide an effective therapy.

Consistency
Many cell types used in cell therapies, such as hematopoietic stem and progenitor cells (HSPCs) and mesenchymal stem cells (MSCs), come from inherently heterogeneous populations. However, it is critical to produce a homogenous cell population to create robust and consistent cell therapies. Induced pluripotent stem cells (iPSCs), for example, require consistency to achieve scalable undifferentiated maintenance and expansion. Homogeneity and reproducibility are directly linked to the formulation and the quality of the media inputs.

”Three key components that drive media optimization are: the product, the cell line, and last but not least the design of the manufacturing process.”

Maverick Lau, PhD
Technological Consultant
Cell Culture Media, Sartorius

Considerations By Stage

Process Development

Developing a cell culture process (for non-cell-based therapies) starts with the creation and selection of a cell line and a suitable clone. Then, the media and process parameters are optimized, typically at a small scale, to facilitate high throughput screening.

During development, biomanufacturers will be searching for the appropriate medium to support a high-performing clone that is able to recapitulate all the CQAs required. Media development can be time- and labor-intensive and could involve several approaches, including spent media analytics, high throughput screening, and single component titration, coupled with testing different feed strategies. Developers may benefit from a DoE approach to assess the maximum number of parameters in the fewest experimental runs. Maximizing the performance of the media during production helps in the downstream purification process, as fewer cells producing more protein means there are fewer host cell proteins and other impurities to remove.

Cell Viability

Cell viability is strongly affected by media, especially for more sensitive cell lines. An important consideration, particularly for standard fed-batch processes, is how many days the medium can support viable cell cultures. Lactate formation and ammonia accumulation are two important phenomena that occur in the bioreactor and could have a significant impact on cell viability – if they are too high, they can be toxic to cells. This is where good process monitoring can benefit the search for suitable media. Solutions such as BioPAT® Trace and BioPAT® Viamass can help you monitor the amount of metabolites and viable biomass in the medium in real-time. This data empowers process development scientists with the information required to fine-tune cell cultivation steps.

Feed Strategy

The way the cells use the media will be highly different depending on whether fed-batch or perfusion feed strategies are used. In fed-batch production, all the nutrients are replenished intermittently to support high density and long-duration processes, whereas, in perfusion culture, the media is continually exchanged. These differences have been shown to impact cell behavior, yield, and the presence of post-translational modifications[7]. For difficult-to-express or highly sensitive molecules, perfusion culture might be the only option.

Additionally, the feed strategy strongly affects the media preparation and storage approach, which will impact the overall facility set operations[8]. Media can be prepared in-house from powdered formula or sourced as a liquid formulation. Additionally, some additives have a short shelf-life and should only be added shortly before use. These features must be carefully considered within the context of the feed strategy.

Design of Experiments Approach

DoE is a systematic approach to performing experiments in a streamlined and cost-effective way. Following DoE principles allows scientists to assess the maximum number of culture parameters using minimal resources. In contrast to a 'one-factor-at-a-time' approach, it reveals interactions been multiple factors to deliver more precise insights, eliminating any guesswork.

Scientists can use multi-parallel bioreactor systems with integrated DoE software (e.g., the Ambr® 15 microbioreactor system with MODDE® software) to apply DoE principles to media development activities. This strategy enables high-throughput screening of various media components and supplements.

Spent Media Analytics

As well as real-time monitoring, services exist that provide detailed insights into the composition of spent media. These insights answer questions regarding how the cell uses the nutrients in the media and what byproducts are released. There are likely opportunities for improvements in the media composition or the feed strategy that can maintain the cells in more optimal conditions throughout the cultivation process, resulting in productivity and product quality improvements.

Deep Process Insights

Real-time process monitoring provides incredibly valuable information during process development. Having these tools in place while searching for the optimal media and culture conditions allows users to unlock previously concealed insights, arming them with new data that can support better decision-making.

This could include taking advantage of process analytical technologies (PATs), which can provide real-time monitoring of critical process parameters (CPPs) and how they interact with different media and feed strategies to influence product yield and quality. This knowledge supports better optimization and control of the process during scale-up and commercialization, where reproducibility and productivity are vital.

Developing a Platform Process

Cell line-specific optimization of media composition and feed strategy will likely generate the best results in terms of product titer. However, hefty pressure to reduce time-to-clinic and time-to-market means that performing comprehensive media development activities for each cell line is not always viable. This factor is particularly important for multi-product facilities and contract manufacturing organizations (CMOs) which may be working with multiple cell lines across various processes.

The reality is that significant optimization work (which can be incredibly time-, cost-, and labor-intensive) may only lead to marginal improvements in the yield. A more efficient approach to accelerate development times could be to develop a "platform" approach for optimizing cell culture processes[9]. This platform should broadly support multiple cell lines, allowing them to perform sufficiently without significant optimization activities[9].

Cell Culture Media Consultancy

An alternative approach to better understand your cells and media needs is outsourcing to a company with the tools and expertise to deliver in-depth insights. This can be particularly helpful when cell viability or product yield is not reaching the expected manufacturing goals, or a cell line is sensitive to changes to its growth environment.

For example, Sartorius can screen a library of media variations to see which combinations work best with your cell type to maximize viability and productivity. Excellent results have been observed with this approach, with a 30% increase in productivity following fine-tuning of media composition.

Custom Media Service

Sometimes luck determines whether an ideal off-the-shelf media is found quickly. Where possible, media selection should form part of the cell line development process during clone selection and process development. However, this is not always feasible in the biopharmaceutical landscape, where speed and efficiency trump the need for comprehensive development activities. Additionally, manufacturers may struggle to adapt their cells to suitable off-the-shelf media, and a more tailored approach could be necessary.

Seeking out a custom media service could be a potential solution for such circumstances. Custom media services typically help you develop optimized media formulations tailored to your cell line and process, resulting in the best possible outcomes for your cells. However, custom media is usually significantly more expensive than off-the-shelf media, and the cost (and vendor capacity to carry out the project) will depend on the batch size. Thus, this approach is not always feasible.

Commercial Manufacturing

Those already in commercial manufacturing are at a different stage in their media journey and will likely have distinct needs. Now that their process has been developed, optimized, and scaled up, manufacturers likely already have key information about their cells' characteristics and media requirements at this stage. They may have implemented an off-the-shelf media that works for their process or selected a custom formulation for a more tailored approach. Their challenges now primarily relate to how they can secure an adequate and high-quality supply of their media to ensure a robust and reproducible manufacturing process.

Assurance of Supply

Building a robust supply network is essential to establishing a reliable bioprocessing pipeline. A 2022 survey reported that "increased focus on supply chain security" was the top operational change impacting biomanufacturers[10].

A media provider with global capabilities will help strengthen the supply chain. Similarly, dual sourcing your medium from more than one supplier can form part of an effective risk mitigation strategy by creating redundancies within the logistics network. Those using custom media must ensure they obtain their own release criteria formulations to help them manage their own supply and build resilience into their supply networks.

Quality and Consistency

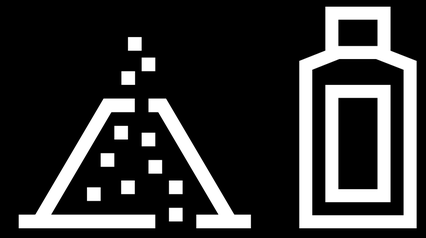
Trace element impurities present in the media can hamper media performance and ultimately affect the quality of your product. Therefore, the quality of the raw materials making up the media components is critical. Choosing a media supplier with systems in place to ensure raw materials are sourced from qualified suppliers and properly characterized to meet high standards can help minimize inconsistencies between media batches.

Consistency between media batches is essential for creating a reproducible process and limiting lot-to-lot variation. Eliminating batch-to-batch variability is particularly challenging if the media contains serum or hydrolysates but is more manageable in chemically defined media, where each component should be well-characterized and subject to quality testing (Figure 2).

Safety

Linked to quality and consistency is meeting regulatory requirements for patient safety, which also requires consistency and traceability across the process. Ideally, all products should be manufactured following GMP and meet regulatory standards from the beginning to limit the need for process modifications later down the line.

Media that minimizes the production of host cell DNA and protein is always favorable. While it might not be a priority during the development of the upstream process, it will significantly simplify your downstream process and avoid regulatory hurdles when demonstrating the safety and purity of your product.



Powder or Liquid?

Typically, powder formulations that are reconstituted in-house are ideal for simplifying transport and storage, limiting costs, and improving facility flexibility. However, ease of preparation may be of significant concern if thousands of liters of media need to be prepared regularly. The decision of whether to purchase powder or liquid media will largely be determined by the facility set-up and the importance of cost and time savings in a particular business.

Concluding Remarks – Benefit From a Comprehensive Service

Maintaining high product quality can be challenging if media or cell culture conditions are changed during scale-up and commercialization. Therefore, defining an optimal media strategy during process development is essential.

Although advances in cell culture technology in robust vector design and host cell engineering methods have led to high yield recombinant protein production, media development remains full of potential for productivity enhancement. A synergistic approach using a defined combination of supplements at specific times of addition is key to improving recombinant protein productivity.

The aim of any media development process is to design a formula and feed approach that achieves high titers and meets product quality requirements. The interactions between the process parameters, cell line, and media ultimately dictate the yield, and product CQAs are challenging to dissect. Significant expertise in all the pillars of cell line development - as well as downstream process steps - is required to make well-informed decisions about how best to optimize your cell culture media.

Choosing a media supplier or service provider that offers solutions through the whole media pipeline supports an excellent media development process. A partner with end-to-end knowledge and expertise can support effective risk mitigation, accelerate timelines, and reduce challenges during future scale-up and commercialization.

Ideally, media development (whether carried out in-house or externally) should be performed during cell line development. If seeking support from a media development service, they should be involved as early as possible in the process, to avoid 'retrofitting' the media strategy to what is already in place, which could result in wasted time, greater risk, and less than optimal results.

The above scenario is not always possible. However, this does not mean it is too late to renew your media strategy and enhance the performance of your cell line. For example, Sartorius provides a flexible high throughput screening service for media development that can help you regain control of your cell line and determine the best composition and feed strategy.

In fact, Sartorius is an excellent example of a supplier with end-to-end solutions. With a comprehensive cell line development platform and service, cell culture instruments, diverse catalog media portfolio, custom media capabilities, process analytical technologies, DoE and quality by design (QbD) software, and spent media analytics services, Sartorius can support you at all stages of your media development journey, helping you maximize productivity and maintain high quality.

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Mayank is part of the Cell line, Media and Testing Solution technical team, where he is a Product Specialist supporting the Cell Culture Media division. He is working on the regional execution of Sartorius' global cell culture media strategy, providing the application services team with technical support on cell culture media for recombinant protein and vaccine applications.

Before joining Sartorius in 2020, Mayank grew his experience as a research scientist in several reputed biopharmaceutical organizations, specializing in the field of cell line development and upstream process development.

Maverick Lau
PhD, Technology Consultant Cell Culture Media, Sartorius

Maverick is part of the Cell Culture Media and Testing Solution marketing team, where he supports technical teams in the efforts of detailing the specifications of products and services.

Before joining Sartorius in 2020, Maverick collected his experience in different research positions in a biomedical research laboratory in the field of respiratory and cancer|inflammation immunology and various industry contracted work. He led two Australia National Grant awarded projects.

Katy McLaughlin
PhD, Scientific Content Writer, Sartorius

Katy is part of the Marketing Communications team at Sartorius, where she supports the creation of a variety of written pieces, from published articles to web content.

Before joining Sartorius in 2021, Katy was employed as a Post-Doctoral Research Associate at the University of Edinburgh, where she also completed her doctoral studies. Here, she carried out research in genetics and cellular biology and began taking on writing projects, eventually entering into a career as a freelance writer for various biotech companies and agencies.

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