Reducing animal-origin components to maximize AAV process sustainability

As a result of their potential as powerful new treatment options, gene therapies are in increasingly high demand, with regulatory bodies expediting clinical and regulatory pathways to accelerate their speed to market [1]. Although timelines are being compressed, it is still important for manufacturers to think about the sustainability and future of their upstream processes. By considering the long-term feasibility of their workflows, manufacturers have a greater chance of maintaining productivity and preventing later process changes, which can be costly and time-consuming.

When it comes to sustainability, many gene therapy manufacturers agree that reducing or completely removing animal-origin (AO) components is an effective way to secure the longevity of their process. In addition to the potential short-term challenges surrounding AO materials, such as lot-to-lot variability and the regulatory hurdles that may arise as a result, manufacturers may also be concerned about possible long-term supply challenges that could increase the risk of costly production stoppages. Fluctuations in the price of AO materials can also make it challenging to predict the long-term economic feasibility of manufacturing processes. Therefore, by considering where they can reduce the use of AO components in their workflows, gene therapy manufacturers can develop processes that are more likely to remain sustainable.

Considering serum use for viral vector manufacturers

Serum is well-established as a supplement for the culturing of lentiviral vector (LV)- and adeno-associated viral vector (AAV)-mediated gene therapies, especially in processes that utilize adherent cell lines. However, even compared to other AO components, its use can be particularly challenging for manufacturers looking to optimize and scale their processes. As a by-product of the meat industry, serum supply is inherently tied in to the dynamics of the cattle cycle. Herd numbers can be influenced by a wide range of factors, from the economics of the global meat industry to environmental variables such as weather and climate patterns. This means that the amount of available serum—and therefore its price—can fluctuate from season to season. As the cost of serum is volatile compared to other raw materials, it can be difficult for manufacturers to predict how this might affect their process in the long term.

In addition to the seasonal fluctuations in price, the overall cost of serum is increasing [2]. Reduced global beef consumption, combined with improved cattle breeding techniques, has resulted in fewer opportunities to extract fetal bovine serum (FBS), which is driving down the available supply. On top of this, demand for serum is increasing as emerging bioprocessing markets continue to grow and new therapeutic modalities are developed. Though some of this demand is being met by a greater availability of newborn calf serum (NBCS), this type of serum comes with its own challenges and presents a higher risk of adventitious agents that can lead to characterization and regulatory hurdles. Furthermore, as the viral vector industry continues to grow and utilize more serum, increased competition may lead to shortages in supply and thus higher costs. This means manufacturers will need to carefully assess the rising price of serum as, over time, the use of large volumes could begin to impact the overall economic sustainability of their process.

A further complexity of using serum in viral vector manufacturing workflows is the need for testing and qualification. As an animal-derived product, serum is not completely defined and can vary from lot to lot. Therefore, it is necessary to regularly test and qualify multiple lots to make sure they fit within the parameters required for a consistent process. This can be expensive, time-consuming, and labor-intensive—particularly if lots need to be disqualified. Beyond this, the quality of sera must be carefully monitored and controlled to prevent the cross-contamination of adventitious agents—including viruses and endotoxins. As a result, it is important to choose a vendor that carefully monitors and audits their supply of sera. For these reasons, manufacturers should consider how reducing or removing serum from their workflow could allow more efficient use of resources and make the process more economically feasible and more sustainable for the long term.

Strategies for reducing or removing serum

It may not be possible for a manufacturer to remove serum from their workflow entirely, particularly if they have developed and optimized a process for producing viral vectors using an adherent HEK293 cell line. Adapting cells to suspension—or switching cell lines entirely—can be labor-intensive, requiring further rounds of process development and optimization. However, by using various supplements and additives, it is possible to make progress toward a serum-free process. For example, peptones are nutrient-rich hydrolysates that are ideally suited for supplementation in mammalian cell cultures. They are available in animal origin–free (AOF) formats and can be combined with the necessary adhesion factors for adherent processes. Peptones can also be used in suspension cultures, which also makes them ideal to boost cell growth if adaptation of a cell line is being pursued.

Utilizing the latest developments in chemically defined media

If manufacturers want to fully eliminate the use of AO components, it is now possible to use chemically defined (CD) media at each stage of the viral vector workflow. Recent advancements in cell culture media for microbial fermentation have led to the development of fully CD production media suitable for plasmid production. Traditionally, plasmid production has been conducted using *E. coli* cells cultured in Luria-Bertani (LB) broth, which consists of a combination of sodium chloride, peptones, and often additional animal-derived components. However, the industry has been trending toward a reduction in AO components for bacterial fermentation, which has resulted in the development of CD media that offer the same nutrition, buffering capacity, and sterilization flexibility, with greater consistency in plasmid production [3].

Furthermore, developments in CD media for HEK293 cell-based viral vector production have led to increased levels of productivity as well as improvements in process consistency. To meet the growing demand for gene therapies it will be crucial to continue maximizing viral yields. Because a robust production process is strongly linked to the quality of the media used, optimizing the desired media formulation will play a key role in maintaining process productivity going forward [4]. By choosing a CD medium—free from serum and other AO components—manufacturers can simplify this optimization procedure and expedite their path to commercialization.

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Other considerations for process sustainability

Beyond the media, supplements, and strategies that a manufacturer can use to reduce or remove AO components, there are wider considerations necessary to maximize the sustainability of their process. Most important is choosing a supplier that they can trust. Maintaining production and avoiding costly stoppages is key for the longevity of any workflow, so it is crucial to partner with a vendor that can deliver reliably, has strong supply chains, and offers manufacturing redundancy and facility harmonization. It is also important to consider whether the current process can be scaled up sustainably in any current or future facilities. Scaling out an adherent process may be viable in the short term, but if production is predicted to increase significantly, then it may make sense to transition to a suspension process. This would reduce the production footprint and could potentially allow limited facility space to be utilized more efficiently.

Ultimately, any optimization decisions should be made as early as possible within process development to maximize the longevity of a vector manufacturing workflow; reducing or removing AO components from a process can be a simple and powerful way to do this.

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