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GMPs-Grade Retroviral Vector Manufacturing: *Process and Facility Design*

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The Good Manufacturing Practices (GMPs) are becoming more and more familiar in biotechnology, and concepts such as quality assurance or validation are now part of the background of clinicians and researchers involved in clinical trials. A recent European Community directive (2001/20/CE) states that GMPs should also be applied to investigational medicinal products and not only to products on the market.[1]

Vector supernatant is a so-called Active Pharmaceutical Ingredient (API) and is subject to the same guidelines as traditional drugs produced by the pharmaceutical industry. This has a deep impact in the field of gene therapy because clinical trials are often run by small biotech companies or, at least in the first phases, by academic centers. The field is continuously developing and, according to the progress of the clinical studies, new processes are necessary to produce large-scale amounts of vector supernatant in a safe and reproducible way.

When considering manufacturing of viral vectors, too many times the term "manufacturing" is used as a synonym for "production" or "production and quality control." In a recent Guidance for Industry issued by FDA, the term "manufacturing" is defined to include receipt of materials, production, packaging, repackaging, labeling, relabeling, quality control, release, storage, distribution of an API, and related controls.[2] According to the definition above, manufacturing of GMP-grade vectors is a complex process that involves different departments (production, of course, but also quality control, quality assurance, logistical, and technical services) and people with different backgrounds and expertise. Important but sometimes overlooked is the relationship between the layout of a facility and the manufacturing method. This is crucial because gene therapy has no standardized production methods and often research laboratories must redesign their spaces in order to be in compliance with GMPs.

Table 1. Airborne particulate classification

	At rest		In operation	
Grade	Max permitted nr of particles/m^3 equal or above			
	0.5 µm	5 µm	0.5 µm	5 µm
A	3,500	1	3,500	5
B	3,500	1	350,000	2,000

FDA's Guidance for Industry dedicates a specific section to buildings and facilities. Buildings and facilities should be "located, designed, and constructed to facilitate cleaning, maintenance, and operations as appropriate to the type and stage of manufacturing." The concept is very clear but not easy to apply for a small biotech or research institute. It is important to emphasize that vector production methods are still being developed. Until a few years ago, the need for large-scale amounts of supernatant was not a priority. Today, a single batch of supernatant has to pass several quality control tests before final release. These tests, especially *in vivo* tests, are very expensive and can represent 80 percent of the cost of a single batch. Moreover, the number of gene therapy clinical trials in Phase II and Phase III is increasing, as is the number of patients involved in a single clinical phase. Therefore, for economic and clinical reasons, different methods of viral vector production are now being developed. Because retroviral vectors are still the most used viral vectors in clinical trials, this article will discuss retroviral vector production and analyze its relationship to the layout of manufacturing areas. Although these considerations may be obvious to people working in big pharmaceutical companies, gene therapy trials often start in academic or research laboratories. In addition, GMPs are required from the beginning of clinical development when an investment in a tailor-made manufacturing area could be a risk.

Because there are no established methods for production of retroviral vectors, both biological and technical aspects have to be analyzed for each kind of vector, keeping in mind that the final goal is an economical, easily reproduced, high-yield process. The first factor to consider is the appropriate packaging cell line: A cell line able

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to grow in suspension can, for example, facilitate further scale-up of the production process in the bioreactor, but its influence on productivity must be carefully analyzed. The composition of the media that supports the selected cell line's growth is another important factor. According to GMPs, the composition and origin (human, animal, or chemical) of the reagent is relevant in terms of safety and quality control.

In addition to these biological aspects, many technical points must be considered. The most important ones are (1) choosing the reactor for the large-scale production of the virus and (2) the ability to establish and control cell expansion and production conditions. Many production possibilities are available, from cell factories to roller bottles to bioreactors with different configurations.[3] The systems derived directly from laboratory scale (e.g., cell factories and roller bottles) are easy to use because they do not require special facility services, such as steam for sterilization, and can be set up into incubators. On the other hand, system monitoring is limited and scale-up and optimization possibilities are reduced. If the producer cell line is able to grow in suspension, traditional fermenters derived from fermenters for bacteria, with minor modifications, are always an interesting possibility. This takes advantage of a technology with a long tradition in the pharmaceutical industry, but costs are high and running a fermenter requires specialized personnel. Other alternatives are available, including hollow fiber and CellCube systems.

In the specific case of retroviral vectors, encouraging results have been obtained from packed-bed bioreactors. In this cell-culture system, cells grow in a basket containing polystyrene disks set up in a vessel very similar to that of a standard fermenter. Media is forced through the basket, thus feeding and oxygenating the cells. The main advantages to packed-bed bioreactors are (1) their ability to use the standard control system of traditional fermenters and (2) their flexibility. In fact, they are suitable for both adherent and nonadherent cell lines and can reach a high ratio between the number of cells and the volume of media, thus allowing a concentration of the retrovirus. The ability to work in perfusion during the cells' growth phase increases the growth rate and avoids concentration of toxic products derived from the metabolism of cells. Moreover, this system traps cells in the basket so separation of the vector supernatant from the cells is relatively easy compared to other systems. Of course, packed-bed bioreactors also have disadvantages. First, it is not possible to take samples of cells during cultivation. Only indirect methods, such as monitoring lactate and glucose levels, can be used to provide information about cell conditions and actual growth rate. The packed-bed bioreactors often require a steam generator for *in situ* sterilization, except for smaller models where it can be done in an autoclave. Moreover, specialized personnel with expertise in fermentation rather than small-scale laboratory techniques are required, and it is not easy to involve people with this expertise, particularly in academic laboratories.

Whatever method of retroviral vector production is selected, the characteristics and location of production spaces are critical for GMP compliance. Many books have been written about the design and construction of new GMP facilities. However, when working with gene therapy clinical trials, it is often necessary to turn a laboratory into a production unit on a limited budget. A complete and detailed list of the specifications of a GMP production area can be found in *EC Guide-Annex 1*, "Manufacture of sterile medicinal products," in which the concept of a clean area (the area dedicated to manufacturing) is introduced and discussed.[4] Clean areas are classified into four grades: A, B, C, and D, with A representing the cleanest. This classification combines the maximum number of viable and nonviable particles allowed to guarantee the safety of the operation. Handling and filling aseptically prepared products, retroviral vector supernatant in this case, should be done in a grade A environment with a grade B background. Tables 1 and 2 summarize the limits for particles in both A and B areas.

It is important to emphasize that all limits refer to operating conditions, which means that the cleaning characteristics for a given area have to be maintained even during routine activities. Most researchers are familiar with a grade A environment; laminar flow boxes, for example, are used routinely in cell biology laboratories and at hospital blood banks. From a technical point of view, building a grade B area is not a problem: clean rooms have been present in the pharmaceutical industry for many years and are used in the electronics industry for microchip production. However, grade B areas are quite expensive to build and maintain both in terms of daily environmental monitoring and in terms of the management required (for example, special training and specific cleaning programs).

Running a GMP manufacturing facility can be difficult at the beginning of a clinical trial, especially for laboratories that are not part of big pharmaceutical companies. Effectively integrating the manufacturing process with the facility design can improve the management of these activities and allow successful evaluations during formal inspections. The technology, quality standards, and existing knowledge must be carefully analyzed and each choice evaluated according to the indications of the regulatory authorities. But GMPs are not a rigid

Table 2. Limits for microbiological monitoring

	Limits during operation			
Grade	Air sample cfu/m^3	Settle plates (Ø 90mm) cfu/4 hrs	Contact plates (Ø 55mm) cfu/plate	Glove print cfu/glove
A	<1	<1	<1	<1
B	10	5	5	5

system of rules; novel solutions can be proposed and accepted by authorities if appropriately justified and validated. Moreover, different solutions can be applied during scale-up after technological improvements have been made. This article analyzes the production of a retroviral vector using a bioreactor at different stages of scale-up, and its impact on facility layout.

Figure 1 shows a typical flowchart for a retroviral vector supernatant production campaign. After the seeding of the bioreactor, an expansion phase follows until, for example, glucose consumption indicates that the supernatant harvest phase can begin. The harvest can be performed in continuous or batch mode. At the end, the supernatant will be filtered, first to eliminate producer cell fragments and then for sterilization. Finally, it will be placed in suitable containers and put in quarantine until the end of the quality control tests.

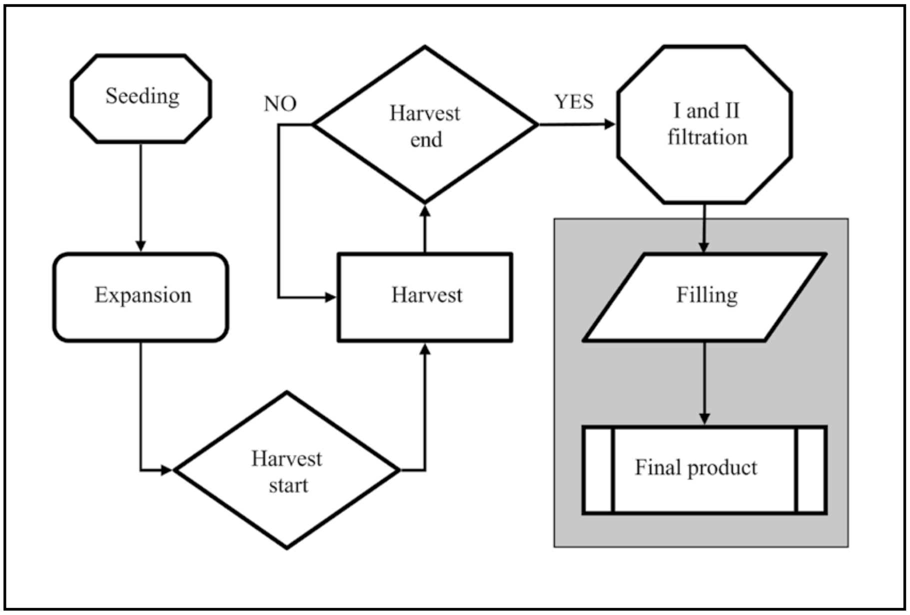

Figure 1. Process flow chart

Retroviral supernatant can be considered a sterile API only after the second filtration; which means that only after this step is a grade A environment with a grade B background strictly required. Therefore, the bioreactor can be set up in a grade C or D area. At the beginning of scale up (in other words, at the beginning of a clinical trial), a final filling unit may already be in place and a pilot plant bioreactor may be in another part of the building. The first critical point occurs when transporting the supernatant from the bioreactor to the filling area; people from production, quality assurance, and logistics must be involved in planning from the very beginning. Initially it must be decided whether to perform the final filtration during the harvest from the bioreactor (on-line filtration) or during the final filling in a grade A environment with a grade B background. In any case, the transport of the retroviral vector bulk has to be regulated by a specific and properly validated procedure. When producing a large amount of supernatant (more than 10 liters), consider using an automatic filling system that is commercially available and can be easily validated. These systems have a lower risk of contamination compared to manual filling, are manufactured in different sizes, and can be adapted to a standard laminar flow box.

In later phases of development, other critical points will be considered. If the trial is running well, a new area dedicated to manufacturing might be planned. In this case, a careful design phase is fundamental. Particularly critical is the flow of personnel and materials, which should always follow separate routes. At this stage, the bioreactors will be set up close to the filling area. Temperature has a great influence on retroviral vector stability. If a continuous mode of operation is established, a cold room should be strategically located for intermediate storage before sterilization and filling. A laminar flow box is no longer necessary in the filling area because bigger automatic filling equipment requires a Laminar Air Flow (LAF) system as used in the pharmaceutical industry.

To conclude, the success of gene therapy trials depends not only on the clinical results, but also on the ability to establish manufacturing methods at reasonable costs that comply with international industry regulations. An effective relationship among people with different expertise — from the lead engineer, to the R&D head, to the quality assurance director, to the researchers and clinicians — will determinate the ultimate success or failure of the gene therapy approach.

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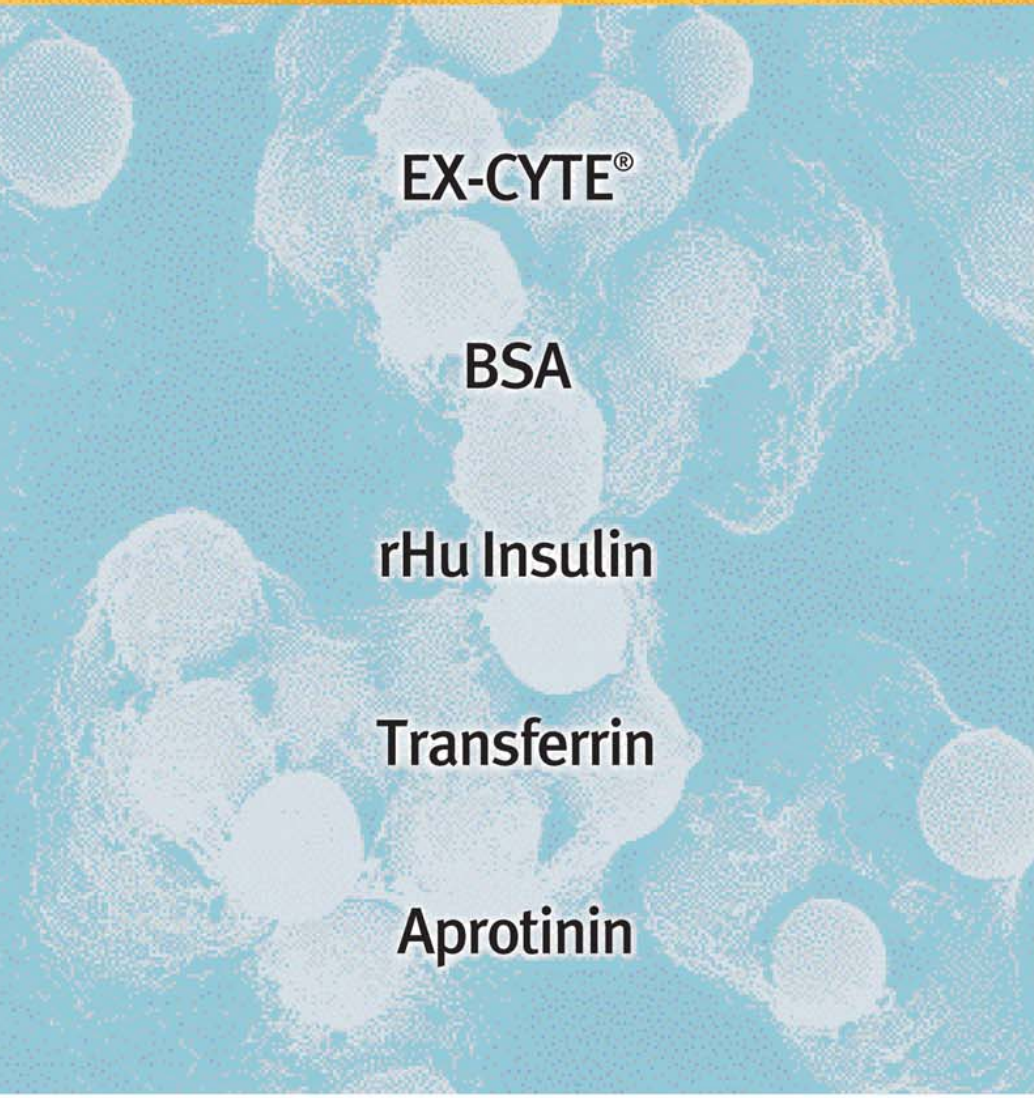

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