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Virus Filtration as an Upstream Risk Mitigation Tool in the Manufacturing of Advanced Therapy Medicinal Products

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Introduction

As the biopharmaceutical industry rapidly transitions to advanced therapy medicinal products (ATMPs), some challenges previously faced with traditional biologics, like monoclonal antibodies (mAbs) and other recombinant proteins, remain equally crucial and must be addressed for these new types of medicinal products.
Despite significant differences between protein-based therapeutics (PBT) and ATMPs, many advanced therapies also rely on cell culture processes or use additional ex vivo cell expansion steps. The use of cell cultures and raw materials of animal or human origin highlights the need for effective virus safety concepts. However, many manufacturing processes for ATMPs lack a standardized downstream process, hindering the implementation of well-established virus clearance steps. Additionally, the size and fragile nature of the ATMP drug substance, e.g., cells or virus particles, further limits the application of certain inactivation or removal techniques. Consequently, the suitability of alternative methods for virus clearance (or their implementation at different stages in the manufacturing process) must be evaluated to ensure virus safety during the production of ATMPs.

Comparison of Process-Related Virus Contamination Risks

Manufacturing processes for ATMPs rely heavily on cell culture processes, e.g., virus particle propagation of adeno-associated virus (AAV) based on HEK293 cell culture, or at least involve a cell expansion step, as applied in most gene-modified cell therapies (Figure 1). Currently, many of these cell culture processes still use raw materials with an intrinsic risk for virus contaminations (e.g., serum to enrich growth media) while also providing ideal conditions for the amplification of adventitious virus particles within the bioreactors.
In the PBT space, overall process safety was dramatically increased due to an industry standard based on rigorous cell line qualifications, careful sourcing approaches, and extensive testing[1]. In particular, active virus inactivation and reduction by various steps in the downstream process have led to a holistic virus clearance strategy that ensures patient safety.
Given that the active drug substance in ATMPs often comprises virus particles (including AAVs and lentiviruses), sensitive cells, or exosomes, many standard virus clearance procedures will eliminate or deactivate the actual active pharmaceutical ingredient (API) without specific differentiation. A further limitation to effectively clear adventitious viruses is the sensitivity and poor process yield of virus- and cell-based ATMPs. This prompts many manufacturing processes to operate without an actual downstream process or to apply only a reduced subset of processing steps.

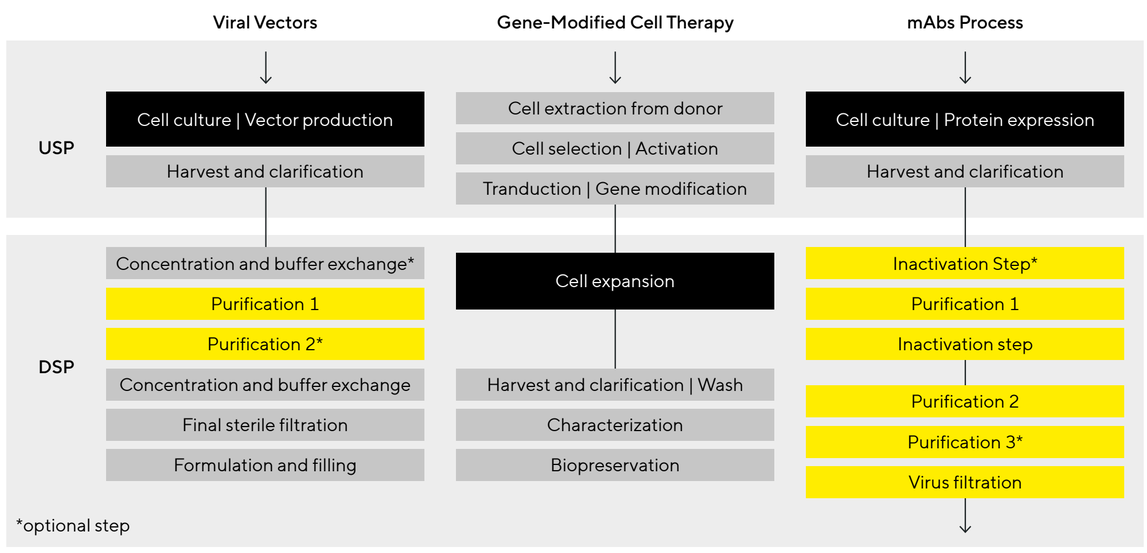


Figure 1: *Illustration of Manufacturing Processes for Viral Vectors, Gene-Modified Cell Therapies, and mAb Production (Simplified).*

Production processes for ATMPs and PBTs rely on cell cultivation processes (depicted in black) using raw materials of animal or human origin, which are potentially associated with virus contamination and propagation risks. Many PBT processes can leverage multiple virus inactivation and removal steps during the downstream process (depicted in yellow) to complement other risk mitigation activities like testing and sourcing. However, the sensitivity of the purification target in ATMP processing often leads to a more simplified downstream purification strategy and reduced opportunities to apply robust virus clearance steps.

Virus Filtration in Upstream Processing

Virus clearance methods applied in ATMP manufacturing are often fortuitous techniques that leverage the virus removal capacity of a unit operation that is already being executed to, for example, capture or polish the drug substance. An example is the separation of AAV particles from virus contaminants and process impurities by affinity capture steps.
Dedicated techniques solely implemented to remove or inactivate adventitious viruses are usually not applied downstream, as they would also act on the actual product and could affect product quality or decrease the yield. Virus filtration is one of the most robust virus clearance methods in downstream processing: While the use of a 20 nm retentive membrane for virus filtration is an industry standard in the manufacturing of PBTs, its implementation in ATMP processes is usually not possible[2].

Nevertheless, the virus removal potential of virus retentive membranes can be leveraged by shifting the position of the unit operation from the downstream to the upstream process using a dedicated filter for cell culture media (Figure 2). Most of the reported contaminations enter the biopharmaceutical process in the upstream process, where media components (including chemicals | salts and glucose) appear to carry a high contamination risk[1,2].
While virus filtration in the downstream processes of traditional biologics is a regulatory requirement supporting virus clearance claims, it also represents a virus risk mitigation strategy when applied in the upstream process[2,3]. Recent updates in the main regulatory guideline ICH Q5A (R2) actively encourage manufacturers to consider complementary actions for virus risk mitigation, especially if active virus clearance in the downstream process cannot be easily implemented or significantly affects process yields[3].

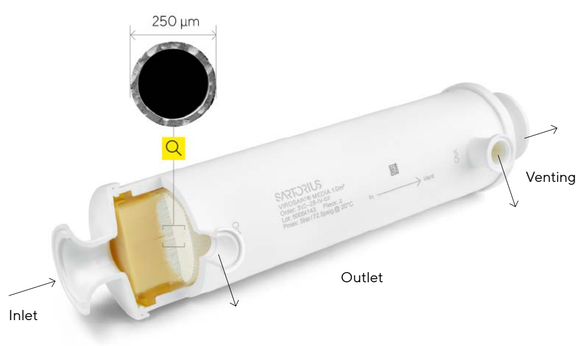


Figure 2: *Design of the Virosart® Media Virus Filter Module. The Compact Packing of the Hollow Fiber Membrane Can Be Seen in the Cross-Section and the Magnification of an Individual Hollow Fiber.*

Risk Mitigation by Virus Filtration of Media

The virus retentive filtration of growth media used for cell cultures or in cell expansion steps represents a powerful tool to reduce the risk of introducing adventitious virus contamination into ATMP processes. As a result, upstream media virus filtration can tremendously compensate for the limited flexibility of these manufacturing processes in the downstream area and increase the overall process safety profile.
Dedicated technologies for upstream applications like Virosart® Media (Figures 2 and 3) are specially designed for virus filtration of chemically defined cell culture media. This high-speed virus filter provides end-users with an economical processing solution while achieving high virus retention with logarithmic reduction values of more than 4 $\log_{10}$, even for small non-enveloped viruses. While the performance is independent of whether powder or liquid cell culture media is used, it can be impacted by the cell culture media itself.
The implementation into single-use processes is enabled by gamma-irradiatable capsule designs and the ability to integrate Virosart® Media into sterile single-use filter transfer sets (Figure 3A) and large-scale filtration formats (Figure 3B).

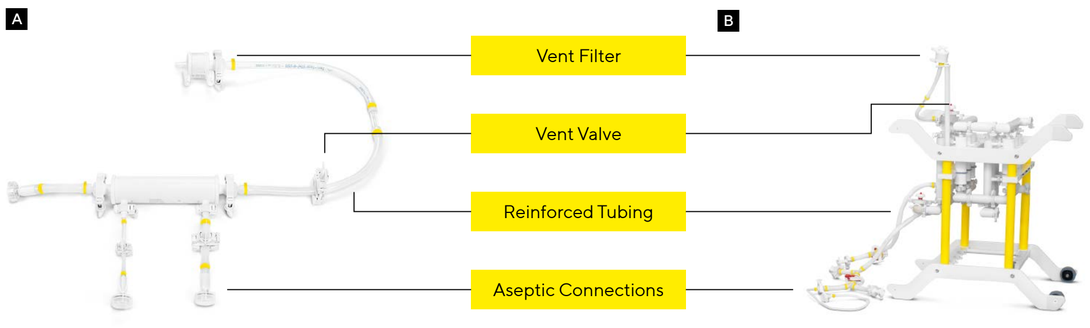
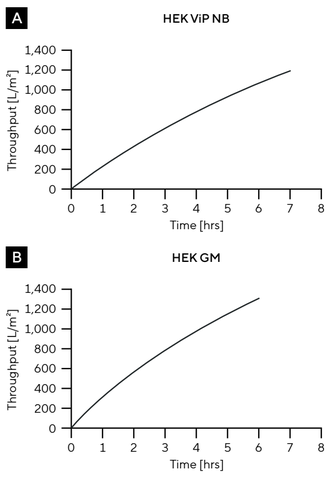


Figure 3: *(A) Sterile Single-Use Virus Filter Transfer Units for Virosart® Media. (B) Sterile, Large-Scale Maxicaps MR® Format for Virosart® Media.*

Adaptation to the required process scale can be achieved using varying capsule sizes and, consequentially, by varying the quantity of built-in virus filter membrane. For example, small- to medium-sized processes can often be realized with individual capsules that can be pre-assembled with all process-relevant connections and pre-sterilized upon delivery. These so-called virus filter transfer units (Figure 3A) enable plug-and-play operations in a sterile manner.
Larger manufacturing processes may require effective membrane areas that exceed the capacity of individual capsules. In such cases, up-scaling technologies like the Maxicaps MR® devices provide the necessary flexibility in commercial production (Figure 3B). These pre-sterilized, large-scale virus filtration devices incorporate the filtration performance of multiple capsules within one functional unit. With this, the efforts required to establish connections and install the filter, potential handling failures, and risks of faulty connections are minimized. Furthermore, all relevant operational steps like venting, flushing, and pre- and post-use integrity testing can be conducted in parallel for all capsules. Such a setup can be seen as a functional equivalent of multi-use filter housings in stainless steel applications, but with the added benefits of single-use technologies. These closed, aseptic systems dramatically reduce the risk of biological- and process-related cross-contaminations during manufacturing, and a true viral barrier can be maintained for processing media into cell cultures.

Virus Filtration Trials for Chemically Defined Media



The benefits of virus filtration for cell culture media stem from its non-invasive mechanism, which ensures reproducibility of the filtered feed streams, as well as simple and cost-efficient scalability. Due to its high robustness, this process step can be applied from small scales during early development stages to commercial manufacturing.
To assess the feasibility of using virus media filtration in the upstream process of ATMP manufacturing, we evaluated the performance of Virosart® Media lab-scale modules using various chemically-defined, serum-, animal component-, and hydrolysate-free media, such as HEK ViP NB and HEK GM. The results indicate that a high flux of more than 100 LHM/bar and total throughputs exceeding 1,000 L/m² can be easily achieved without including any pre-filtration (Figure 4). Importantly, as many applications in the ATMP space utilize cell cultivation steps at smaller scales, it is feasible to achieve filtration of the required process volumes either with filtration areas significantly smaller than 1 m² or in very short processing times, both of which lead to reduced cost contributions in the overall process setup.

Cell Culture Media	Total Throughput [L/m²]	Total Time [hrs]	Capacity in 4 hrs [L/m²]
4Cell® MDCK CD*	719.96	4.4	678.18
4Cell® BHK-21 CD*	999.40	4.3	946.58
HEK ViP NB	1239.48	6.9	802.86
HEK GM	1321.14	5.75	983.39

Figure 4: *Virus Filtration of Various Chemically-Defined Media. The Virosart® Media Virus Filter Was Able to Process up to 1,300 L/m². The Filtration Performance of HEK ViP NB and HEK GM Shows High Volumetric Throughput (L/m²), Operated at 2 bar. (*Protein-Free)*

Conclusion

Virus filtration of cell culture media and additives represents an easy-to-implement risk mitigation strategy to increase the overall safety profile of ATMP manufacturing processes. The application of virus filtration in upstream processing can especially benefit production setups depending on high-risk materials or components that cannot be substituted by safer alternatives.
The data presented in this poster demonstrate that cell culture media commonly utilized in the culture and propagation of cells used in the manufacturing of ATMPs can be effectively processed by virus filtration. Although a focus is often put on animal- and human-derived raw materials when evaluating high-risk materials, it is important to also consider other media components as potential sources of virus contamination. As a result, virus filtration of cell culture media can add substantial benefits by mitigating the risk of virus amplification during cell culture and expansion stages without compromising the product yield.

Refenrences

1. Barone et al., 2020, “Viral contamination in biologic manufacture and implications for emerging therapies”
2. Johnson et al., 2022, “Virus filtration: A review of current and future practices in bioprocessing”
3. EMA/CHMP/ICH/804363/2022 | ICH Q5A(R2) “Guideline on viral safety evaluation of biotechnology products derived from cell lines of human or animal origin”