White paper

Developing an Accelerated and More Cost-Effective Single-Use Adenoviral Vector Vaccine Manufacturing Process through Public-Private Collaboration

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Beginning with Edward Jenner's breakthrough method to protect against smallpox in the 1790s, vaccines have saved and improved millions of lives around the world. UNICEF touts immunization as one of the most cost-effective public health interventions to date, averting an estimated 2 to 3 million deaths every year. Yet while remarkable progress has been made in eradicating devastating diseases such as smallpox and polio, the global population remains at risk due to difficulties accessing vaccines, shortages in supply, slow responses to outbreaks and pandemics and the presence of emerging pathogens. Nearly twenty million children under one year of age worldwide did not receive the recommended doses of diphtheria, tetanus and pertussis vaccine (DTP) in 2017, and a similar number of children failed to receive a single dose of the measles vaccine.

In addition to these well-known pathogens, emerging and re-emerging diseases continue to pose a challenge. Consider the World Health Organization's priority list of infectious diseases for which R&D efforts are needed to enhance our preparedness. Among the top ten is the ominously named "Disease X" – a placeholder for a yet-to-be-identified potential disaster, and emphasizing the need for platform technologies which can be rapidly adapted to respond to any pathogen, without prior knowledge.

Undoubtedly, vaccine manufacturing can be a time-consuming and costly endeavor, requiring a balance of competing goals as described by Plotkin, et al.

> *Emphasis on process development is a major success factor in being first to market with new [vaccines] and inadequate process development is often implicated in late stage product development failures. Manufacturers are challenged to balance the competing goals of speed to market and process optimization; getting to market earlier increases revenue in the short term, but locking in a further optimized process may generate cost savings over the entire vaccine life-cycle.*

We believe that public-private collaboration is essential to drive development of much needed, new approaches to vaccine manufacturing that enable both speed to market and process optimization. In this whitepaper, we describe our collaboration with the Jenner Institute, Oxford University in the United Kingdom to develop an optimized, single-use GMP process for manufacturing adenoviral vector-based vaccines. Founded in 2005, the Jenner Institute is a partnership between the University of Oxford and the Pirbright Institute and is a successor to the former Edward Jenner Institute for Vaccine Research.

Adenoviral Vectors

Adenoviral vectors, used for cancer and gene therapy, offer a number of advantages when used as vaccine delivery vehicles including the ability to elicit both innate and adaptive immune responses. A major drawback of adenoviral vectors, however, results from pre-existing immunity toward the vector. More than fifty serotypes of adenovirus infect humans and more than 80% of the population has been exposed to at least one serotype and developed a serotype-specific immune response. This preexisting immunity against the vector can significantly reduce immune response to the vector's payload (the pathogen antigen expressed from the transgene inserted in the vector).

In recent years, adenovirus vaccine development has explored used of simian-derived adenoviral vectors, which have negligible seroprevalence in the human population. While overcoming the problem of pre-existing anti-vector immunity, they retain the advantages of other replication-incompetent adenoviruses:

- Induction of a broad cellular and humoral immune response against the target antigen
- Safety, with no viral replication in the vaccine recipient due to deletion of essential genes
- Lack of host genome integration
- Efficient infection of a variety of mammalian cell types including antigen-presenting cells
- Compatibility with different processing technologies including stirred tank bioreactors, high capacity filtration methods and chromatographic purification procedures as a result of their neutral charge and relatively small size. This size (c 80-90 nm) is compatible with final sterile filtration (0.2µ) eliminating the need for a validated and cumbersome closed processing approach

Our collaboration sought to advance development of a rapid, scalable and GMP-compliant manufacturing process for simian adenoviral vector vaccines including those targeting common pathogens such as rabies and emerging threats like Zika and Ebola. Our goal was to improve the manufacturing process for adenoviral-based vaccines for Phase 1 materials at a scale of $>5x10^{13}$ VP (virus particles; equivalent to 2000 doses) which could then be readily scalable to $5x10^{14}$ VP. We sought to develop a cost-effective and transferable template with a minimum of 50% overall efficiency that could be used to accelerate vaccine development and manufacturing worldwide. The first candidate evaluated as a pilot was the rabies vaccine.

A more robust template for adenoviral vector manufacturing based on an easy-to-operate and easy-to-implement GMP single-use process has the potential to deliver a number of benefits including:

- Accelerating clinical phases
- Reducing time to market
- Enabling a more rapid response to outbreaks and pandemics
- Meeting the need for more affordable medicines in low resource settings

The New Viral Vector Manufacturing Template

The initial template included labor intensive operations, open and lengthy processing. For the new template we followed the schematic platform in figure 1.

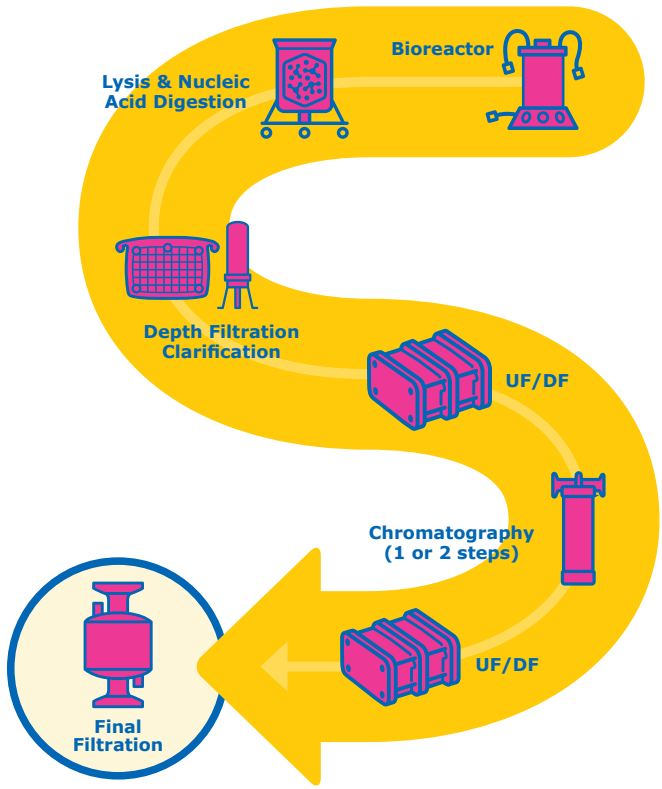


Figure 1. Schematic of a typical adenovirus vector manufacturing process.

The goal of the upstream portion of the process is to grow the cells infected with the virus and achieve the desired target titer. Following the upstream process, the virus is purified by a series of steps, separating it from contaminants and host cell proteins. Once purified, the virus is formulated in a stable manner and the final vials filled.

Key challenges during this process are:

- Achieving sufficient viral titers
- Open handling steps which can lead to increased risk of contamination
- Ensuring maximum virus recovery
- Purification cost and scalability
- Purity

The transition to a new manufacturing template focused on reducing the need for handling and compressing processes in order to evolve away from labor intensive, risk-associated operations. We sought to eliminate shake flasks which must be opened in a manual process, centrifugation because of the associated maintenance, investment and the scalability challenges and ultra-centrifugation.

The new template compressed the process into seven steps and maximizes use of disposable technologies. In addition, we aimed at developing a template that can be used to manufacture different adenoviral vectors and tested two other candidates in parallel to the rabies vaccine.

Upstream

For the new upstream process, Mobius® three-liter and other single use bioreactors were run in fed-batch mode after inoculation with a seed train of HEK293 T-rex. A significant increase in yield was achieved by converting from shake flasks to single use bioreactors (figure 1) Upstream process yields per bioreactor vessel ranged from $7.2x10^{13}$ to $2.5x10^{14}$ VP (qPCR) across one run with ChAdOx2 RabG, two runs with ChAdOx1 RVF, and two runs with ChAd63 ME-TRAP, i.e. >2500 human doses of c. $2.5x10^{10}$ VP.

Vessels	Cells	Cell density at infection	MOI	Time of Harvest	Cell density at harvest	Yield (qPCR)
2 x 3L Mobius® 3L Bioreactors	HEK293/ HEK393 T-Rex	1.5-2 $x10^6$	3	42 Hours	1.2 x 10^6 VP/ mL	~5x 10^{10} VP/ mL

Figure 2. Performance summary of HEK293 culture and adenovirus production in 3L Mobius® Bioreactors.

DNA Reduction

Nucleic acid digestion to meet regulatory requirements and facilitate downstream processes was accomplished using Benzonase® nuclease. Addition of the nuclease at 60 U/mL of cell culture media decreased the level of host cell DNA >1500 fold during the two-hour lysis in the bioreactor, meeting the regulatory requirement of < 10ng/dose. The overall process was compressed as the lysis was performed directly in the bioreactor. As shown in Table 1, lysis was sufficient after only 30 minutes, reaching the target of 10ng/dose; after two hours, the reduction is more than 1,500-fold. In the light of the results, it is likely that a lower concentration would suffice to reach the 10 ng/dose and DNA digestion < 200 base pair requirement. Further optimisation could be performed to achieve sufficient DNA digestion within a satisfactory time using a dose of Benzonase® more suitable for scalability.

Removal of DNA and its impact on viscosity also reduced the potential for blockages and the need for a larger filtration areas in downstream operations.

Sample – Set point	Host DNA ng/mL
Bioreactor – pre-lysis	1850
Benzonase® nuclease addition	1650
30 min	8.6
1 h	2.9
1 h 30 min	1.4
2 h	1.1
3 h 30 min	0.4

Table 1. Reduction in host DNA following treatment with Benzonase® nuclease met the regulatory requirement of < 10ng/dose within 30 minutes.

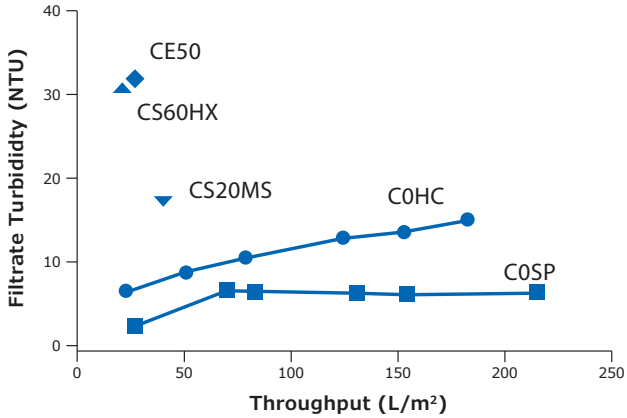
Downstream

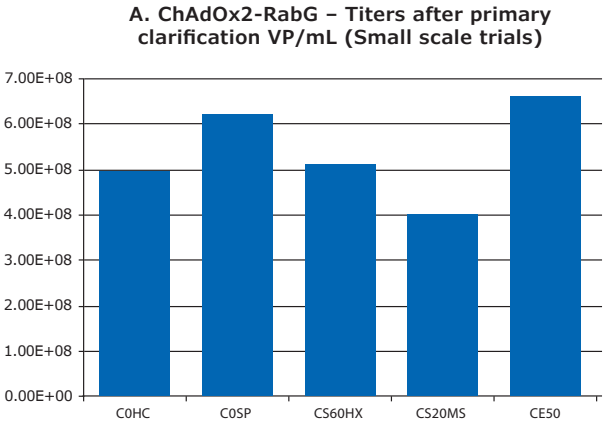
For this new process, we replaced the centrifugation unit operation with clarification using depth filters which are disposable and used in normal flow filtration and thus easy to operate. Because clarification has a strong impact on subsequent downstream steps, we sought to achieve as high a recovery as possible, in this case, greater than 90% along with turbidity reduction.

While adenoviral vectors are neutral, we still wanted to test different filters and select the best option to maximize adenovirus recovery. Table 2 describes the three Millistak+® and two Clarisolve® depth filters that were tested; each were different grades, micron ratings and charge.

Depth filter Name	Media type	Description
Millistak+® filter	C0HC	Double layer, High capacity Cellulose Diatomaceous earth Nominal micron rating 0.2-2µ
Millistak+® Pro filter	C0SP	Synthetic material (polyacryl & silica) Nominal micron rating 0.2-2µ
Millistak+® filter	CE50	Single layer Cellulose Nominal micron rating 0.6-1µ
Clarisolve® filter	CS20MS	Polypropylene fibers Diatomaceous earth Nominal micron rating
Clarisolve® filter	CS60HX	Polypropylene fibers Nominal micron rating

Table 2. Depth filters evaluated for use in the new viral vector manufacturing process.





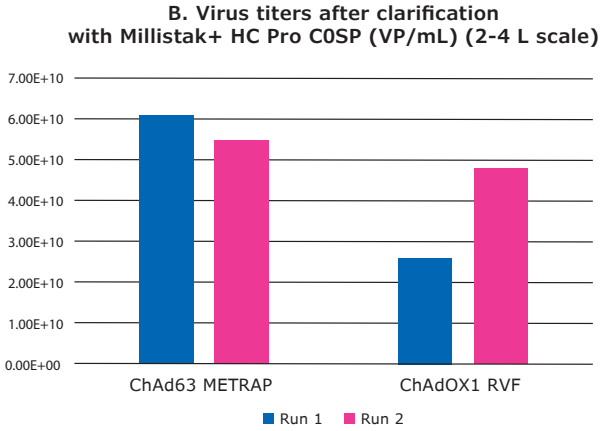


Figure 3. Evaluation of virus recovery using different depth filters. The Millistak+® HC Pro COSP performed best in terms of turbidity reduction and virus recovery.

Figure 4 summarizes our evaluation of the different filters listed in Table 2. Virus recovery was similar for all and achieved our goal of >90% as measured by qPCR. The high capacity Millistak+® HC Pro COSP filter performed best in terms of turbidity reduction throughput; we achieved a single-step turbidity reduction from 70 to 7 NTU Additionally, we were able to process more than 2.3L without reaching a true final end pressure point (Pmax). In the small-scale trials, we actually never reached a true Pmax, which is a constant flux methodology with pressure monitoring. The solution then went through a bioburden reduction step using an Opticap® XL50 0.2 micron filter before moving to purification.

To remove the requirement for ultracentrifugation at this point, we opted for tangential flow filtration. We achieved an 80-88% product recovery and 20-30x reduction in host cell protein contaminants using the following method:

- 300 kDa Pellicon® 2 Mini filter (0.1m^2) with Biomax® membrane
- 5L starting volume after clarification (Millistak+® 10x volume concentration
- 10 DV filtration against IEX-suitable buffer (containing 100 mM NaCl)

A key factor when performing tangential flow filtration, and something we recommend when handling viruses with 'open' membranes (>100 kDa MWCO, potentially permitting high flux and high conversion fractions even with low transmembrane pressure (TMP)), is permeate control. A permeate control is performed with addition of a pump or valve to partially restrict the permeate site to avoid the creation of an uncontrolled polarization layer. Once the TMP is stable, less stress is applied to the viruses and this helps ensure better recovery and reduce fouling (Figure 4).

The objective of the chromatography step was to use disposable membranes instead of resins which must be packed, cleaned and validated. To this end, we leveraged our NatriFlo® HD-Q membrane, a quaternary amine grafted on a macroporous hydrogel-coated polypropylene membrane. This design combines the advantages of traditional resins and absorptive membranes leading to high-capacity and fast flow rates, allowing us to further compress the process and reduce costs. This technology is highly versatile and can be used in flow through or bind-elute modes, with ion exchange and affinity methods. For this process, we evaluated the anionic change version and performed the chromatography in a bind-elute mode using the parameters in table 3.

Loading	Flow rate	Equilibration and wash buffer	Elution buffer
~3.30 x 10^{11} VP	2 mL/min	50 mM NaPhosphate pH 6.5, 5% sucrose, 100 mM NaCl, 1 mM $MgCl_2$, 0.1% Tween20	50 mM tris-HCl pH 8.0, 1M NaCl, 5% sucrose, 1 mM $MgCl_2$, 0.1% Tween20

Table 3: Process parameters for the chromatography step with Natriflo® HD-Q Recon Mini 0.2 mL.

A good elution of the rabies vaccine candidate was obtained with Natrix® HD-Q membrane adsorber in bind & elute mode when loading in a phosphate based buffer (Figure 6 & 7). With the membrane, we were able to achieve a DBC of 6, an 85% reduction in host cell protein along with a satisfactory 76% recovery of the virus (Table 4).

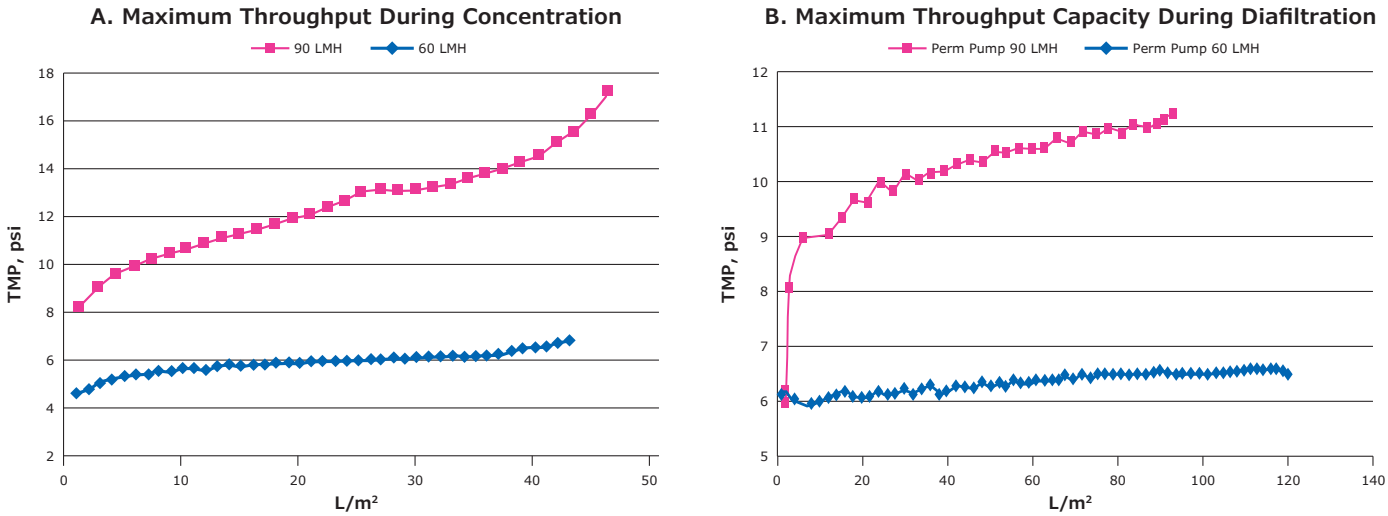


Figure 4: Small scale trials of the Tangential flow filtration experiment with clarified lysate of ChadOx2-RabG using 300 kDa MWCO Biomax® PES C-screen Pellicon® 2 Mini cassette. A. TMP excursion during concentration: B. TMP excursion during diafiltration.

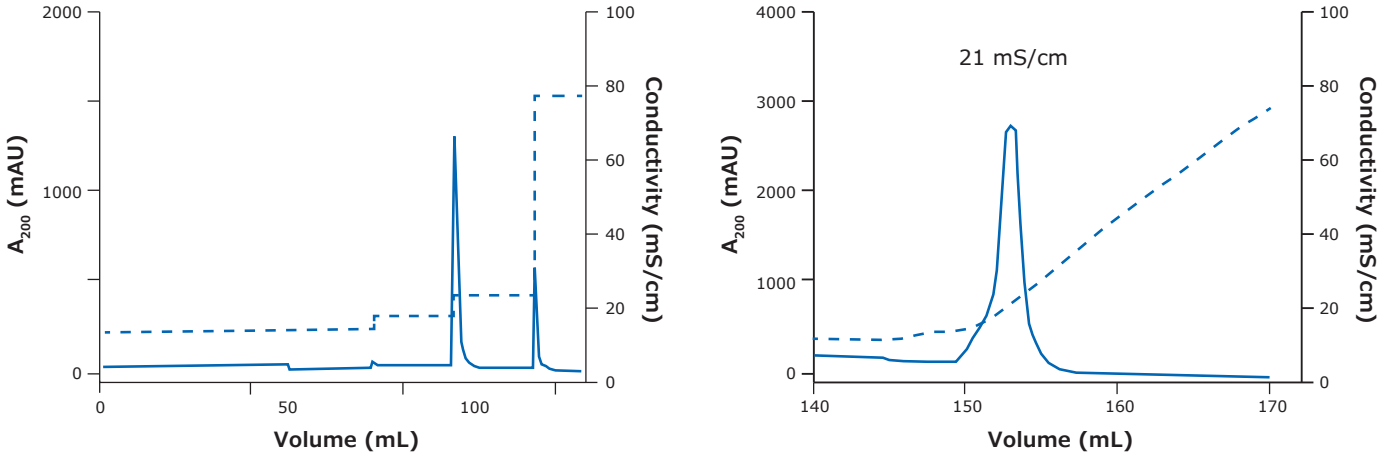


Figure 5: Chromatography of ChAdOx-2-RabG vaccine using Natrix® HD-Q membrane adsorber. (Left) Step elution of ChAdOx2-RabG with Natrix-HD Q membrane adsorber. (Right) Gradient elution ChAdOx2-RabG elution peak at 21 mS/cm.

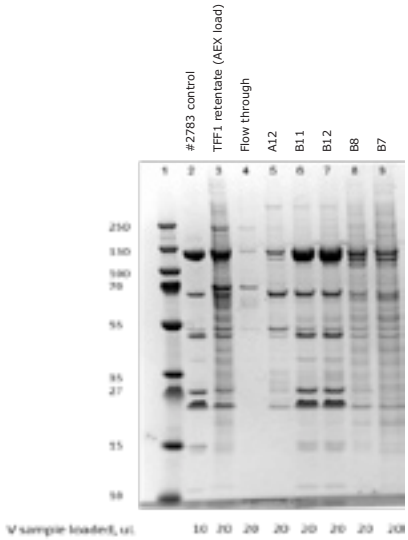


Figure 6. SDS-PAGE of ChAdOx-2-RabG vaccine after anion exchange chromatography. Elution peak samples were loaded in lines B11 and B12.

	DBC (Dynamic binding capacity, VP x 1013/mL bed)	HCP reduction (%)	Recovery (%)
Natrix-HD Q membrane adsorber	6	85	76

Table 4: summarize the Natrix® HD-Q membrane adsorber performance with ChadOx2-RabG.

DBC was defined as the load at which breakthrough A280 exceeded that observed early during loading (i.e. flowthrough A280) by 10% of the difference between the A280 of the loaded sample and the flowthrough A280.

Single use flowpath

A further objective of the work was to rely on single use technologies in the development of the manufacturing platform. pH & DO control can be achieved on the single use Mobius® 3 L bioreactors using commercially available invasive single use probes. The upstream process can be scaled up to a 2000 L Mobius® single use bioreactor, which includes a single use bag with an external loop dedicated to sampling either with multi-use or single use probes.

Rigs for clarification & TFF were designed to ensure a closed process.

Performance Summary

Figure 7 provides a summary of the new adenoviral vector vaccine manufacturing template. This optimized workflow requires approximately five days – two days for upstream operations plus clarification and DNA digestion, followed by three days for the purification, which is solely based on filtration. The new process is a full single-use flow path including bioreactors, tubing and liners, collection bags, filters and chromatography membranes. At the 3L scale, this new process yielded product suitable for early-phase clinical trials, reaching a target of 2000 doses from a 4L batch with an overall process efficiency of 50%. This process can be scaled to 2000L, using different single-use technologies such as bioreactor bags and mixing systems for media and buffer storage and intermediate mixing and storage (Figure 8).

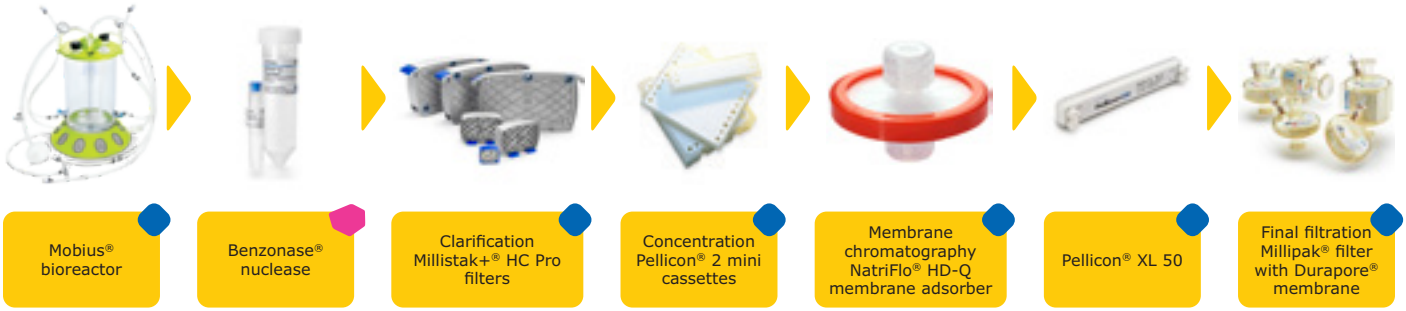


Figure 7. A optimized GMP-transferable process for adenovirus-based vaccine manufacturing.

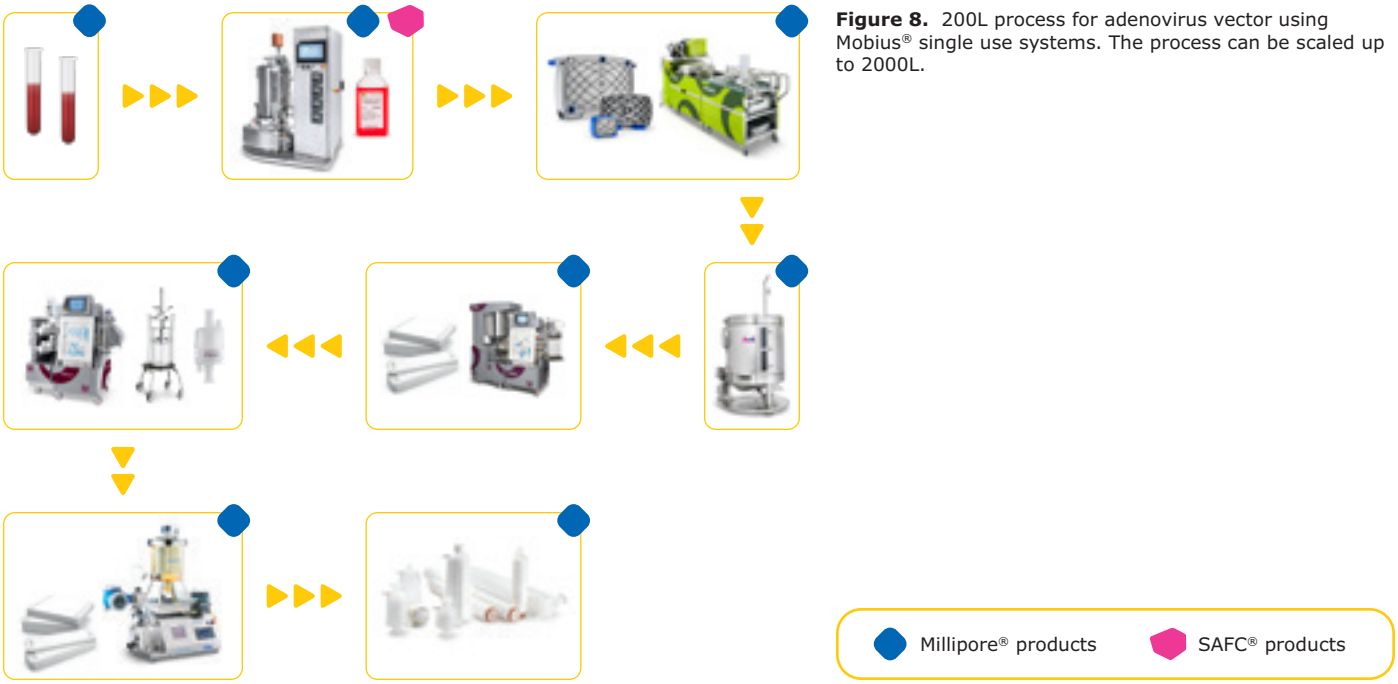


Figure 8. 200L process for adenovirus vector using Mobius® single use systems. The process can be scaled up to 2000L.

Our collaboration with the Jenner Institute has resulted in a single-use, scalable GMP template for manufacture of adenoviral-based vaccines. The new approach compresses the time required to manufacturing doses to within one week (excluding QC time), which is critically important during an outbreak. We met our initial goals of producing at least 2000 doses from a 4L culture with the necessary host cell protein removal and virus recovery. Robustness of this approach has been demonstrated by the Jenner Institute for a number of different adenoviruses.

This successful collaboration is an example of how public and private organizations can come together to solve challenges facing our industry and deliver meaningful solutions to benefit the global population. With this faster and more cost-effective approach to vaccine manufacturing, we take an important step towards expanding access, address shortages in supply and accelerate our response to outbreaks and pandemics.

References

i. https://data.unicef.org/topic/child-health/immunization/

ii. https://www.who.int/blueprint/priority-diseases/en/

iii. Plotkin S, Robinson JM, Cunningham G, Iqbal R, Larsen S. The complexity and cost of vaccine manufacturing - An overview. Vaccine. 2017;35(33):4064–4071. doi:10.1016/j.vaccine.2017.06.003

iv. https://www.intechopen.com/books/adenoviruses/adenoviral-vector-based-vaccines-and-gene-therapies-current-status-and-future-prospects

v. Ahi YS, Bangari DS, Mittal SK. Adenoviral vector immunity: its implications and circumvention strategies. Curr Gene Ther. 2011;11(4):307–320.

vi. Fedosyuk and al. Simian adenovirus vector production for early-phase clinical trials: A simple method applicable to multiple serotypes and using entirely disposable product-contact components. Vaccine. 2019; 37(47):6951-6961

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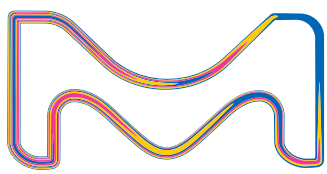

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Lit. No. MK_WP4773EN Ver. 1.0
2019 - 24035
12/2019