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White Paper

Downstream Process Intensification for Virus Purification Using Membrane Chromatography

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Viruses and their components are used in a variety of therapeutic modalities including vaccines, gene therapies and oncolytic applications. Regardless of the intended use, the viruses must be produced via a robust and cost-efficient process. Unlike monoclonal antibodies (mAbs) for which production processes are highly industrialized and well-characterized, the diversity of viruses and processes used for growth have precluded development of a similar templated approach which would help drive cost and production efficiencies.

This white paper provides an overview of the challenges presented by current approaches to virus production and the opportunities to develop a platform approach that can work across different viral modalities and accelerate process development. A series of case studies highlighting the application of downstream process intensification for production of viral vectors, oncolytic viruses and inactivated viral vaccines is provided.

The Challenges of Virus Production

A comparison of the process used to produce viral capsids for gene therapy with that used to produce mAbs immediately reveals key differences (Figure 1). The templated mAb process has a steady cadence of sequential development and clinical phases during which process decisions are made. While continued progress towards key milestones is imperative,

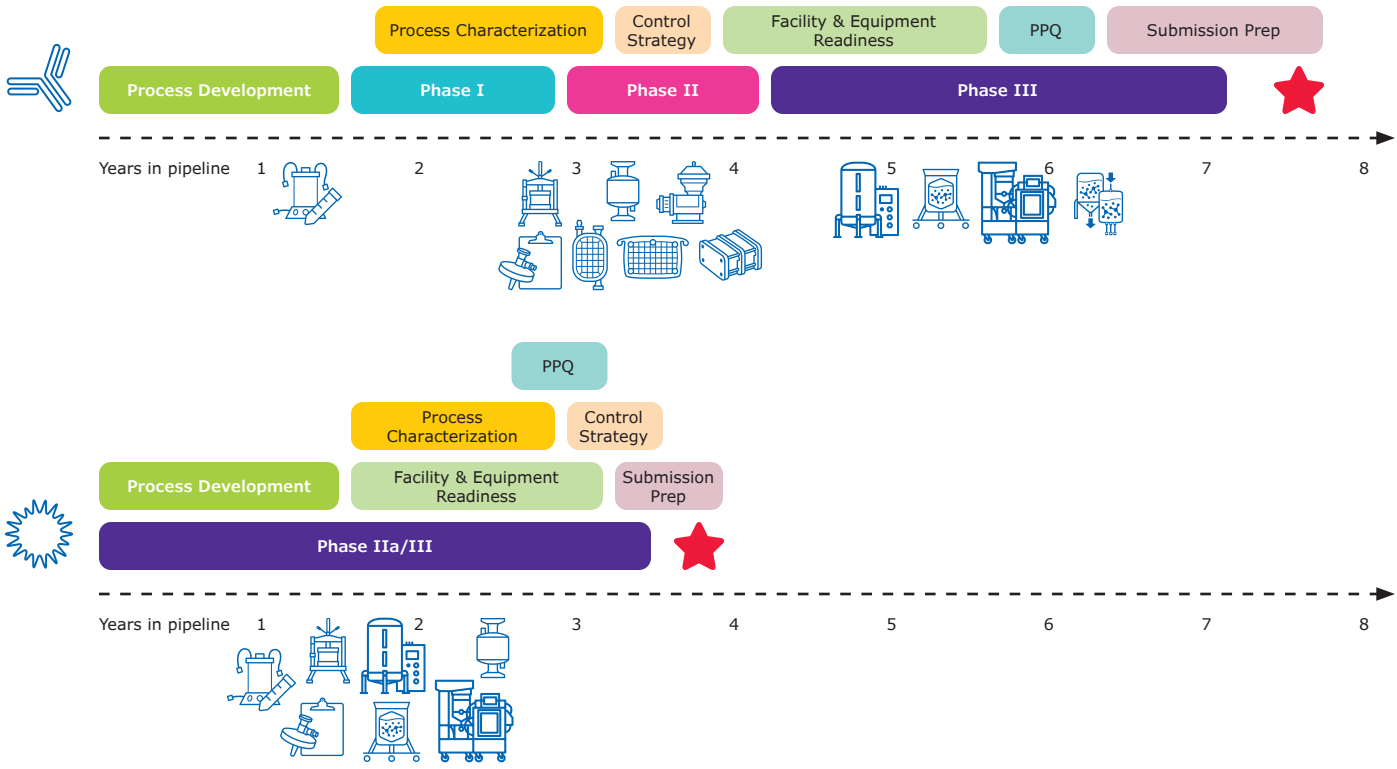
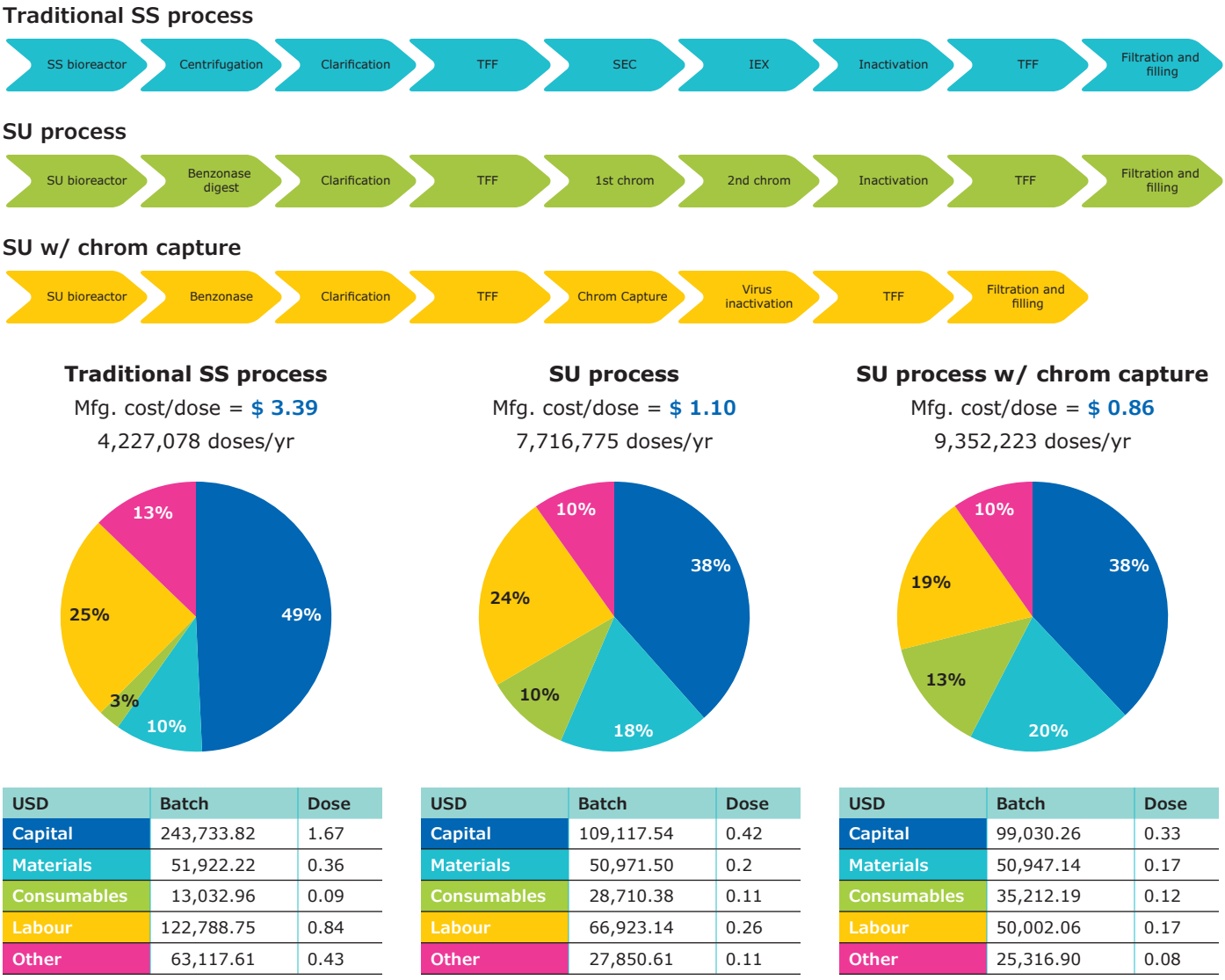


Figure 1. In comparison to mAb production (A), the timeline for development of vectors for use in gene therapy (B) is highly compressed with overlapping steps necessary to meet aggressive timelines.

there is sufficient time to optimize processes as the molecule moves toward and into the clinic. In contrast, clinical timelines are significantly compressed for the development of gene therapies, owing to their approval process, the limited patient populations they target and, in many cases, the dire need for treatment. Many process-related decisions that impact downstream operations must be made early and within a very short period of time. If the resulting downstream process is overly complicated as a result, the pace of development is likely to slow.

Similar challenges exist during vaccine production. In this case, production costs, which translate into the cost of the vaccine, are an area of concern for manufacturers. Process intensification, in which the number of steps and facility footprint are reduced, can be used to reduce costs and accelerate development. The impact of process intensification is shown in Figure 2. Three hypothetical scenarios were modeled: traditional stainless steel with a fairly complex downstream process; a single-use template which uses benzonase digestion and other process improvements; and a third approach in which all chromatography steps are replace by one. The model assumed a 500L bioreactor, two seed bioreactors and an 80 percent facility utilization.

As shown in Figure 2, there was the expected reduction in the capital investment, which dropped significantly with the conversion to single-use. As the simulated process was intensified by compression of the chromatography steps into one, the cost of materials decreased. While the cost of consumables increased slightly with single-use, the cost of labor decreased. With a more complicated stainless steel process, a larger facility is required as well as more overhead and labor, which combines to make a significant difference in the cost per dose. Cost per dose is further reduced with process intensification. Overall, intensification with a foundation of single-use technology leads to a better and faster process by increasing development speed, which translates into smaller facilities and lower costs. Specific advantages include the ability to:



USD	Batch	Dose
Capital	243,733.82	1.67
Materials	51,922.22	0.36
Consumables	13,032.96	0.09
Labour	122,788.75	0.84
Other	63,117.61	0.43

USD	Batch	Dose
Capital	109,117.54	0.42
Materials	50,971.50	0.2
Consumables	28,710.38	0.11
Labour	66,923.14	0.26
Other	27,850.61	0.11

USD	Batch	Dose
Capital	99,030.26	0.33
Materials	50,947.14	0.17
Consumables	35,212.19	0.12
Labour	50,002.06	0.17
Other	25,316.90	0.08

Figure 2. Comparison of the cost of goods sold resulting from a stainless steel process, single-use, or single-use with chromatography capture process.

- Reduce initial capital investment and delay large expenditures when risk is high
- Accelerate process and facility development
- Right-sized process design to reduce the process footprint
- Reduce the time and labor needed for unit preparation and cleaning
- Produce multiple products in a single facility
- More flexibly respond to unpredictable changes in demand

The following case studies describe downstream process intensification for virus production using Natrix® chromatography membrane to increase productivity and reduce capital and operational manufacturing costs.

Structure and Advantages of Membrane Chromatography

In contrast to resin chromatography where the mass transfer is dominated by diffusion and requires long residence time to achieve decent binding capacity, membrane chromatography relies on a conventional flow that enables high flow rate operation. However, the typical highly permeable membrane structures that facilitate very short residence time operations exhibit modest binding capacity due to a limitation of binding site density. Advances in membrane science that have focused on resolving this fundamental trade-off have resulted in affinity and ion exchange membranes with a combination of high dynamic loading capacities with <10 second residence time, opening the way to an intensified high productivity, truly single-use purification platform.

The high binding, short residence times of Natrix® Q chromatography membrane are enabled by the combination of a non-woven reinforcing mesh skeleton and porous hydrogel containing functional groups (Figure 3). The skeleton provides mechanical strength and durability, while the hydrogel creates a large three-dimensional surface area that contains a high density of functional groups with interconnected pores allow for convective flow channels to achieve high flow rates. The high density of binding sites, together with a macroporous structure, enables high binding capacity for not only proteins, but also large molecules such as viruses and DNA. Residence times are on the order of seconds, without compromising binding capacity which altogether enable very high productivity purification processes.

Creating a Cost-Effective and Scalable Vaccine Platform

In collaboration with the Jenner Institute at the University of Oxford, we developed a cost-effective and scalable manufacturing platform for an adenovirus-based vaccine that is suitable for GMP production. The original lab-scale process was labor- and time-intensive and required use of shake flasks and ultracentrifugation to purify the virus. This low throughput, low yield process lacked robustness and would have been difficult and expensive to scale.

Ultimately, the goal of process intensification is to increase the productivity and reduce capital and operational costs of manufacturing facilities. This can be achieved through eliminating, connecting and accelerating unit operations, as well as reducing non-value-added downtime at the plant. For this process, we replaced centrifugation with depth filtration at the clarification step and gradient ultracentrifugation with Natrix® Q chromatography membrane, a salt- and phosphate-tolerant strong ion exchanger (Figure 4).

Although it is a one-for-one exchange of technologies and the number of unit operations were not reduced, the overall productivity of the process is significantly improved, with reduced capital investments at scale. Moreover, the workflow was transitioned to a fully single-use process, drastically reducing the downtime and expenditure associated with unit cleaning, storage, and upkeep.

Tangential flow filtration (TFF) retentate of a vaccine candidate from the adenovirus vector platform was used for evaluation of the new process. Natrix® Q chromatography membrane was loaded to 6 × 10^{13} viral particles per mL membrane. The majority of the virus came off at approximately 22 [mu] / Siemens per centimeter in the gradient elution, corresponding to a recovery of 76%, with host cell protein (HCP) reduced by 85% (Figure 5).

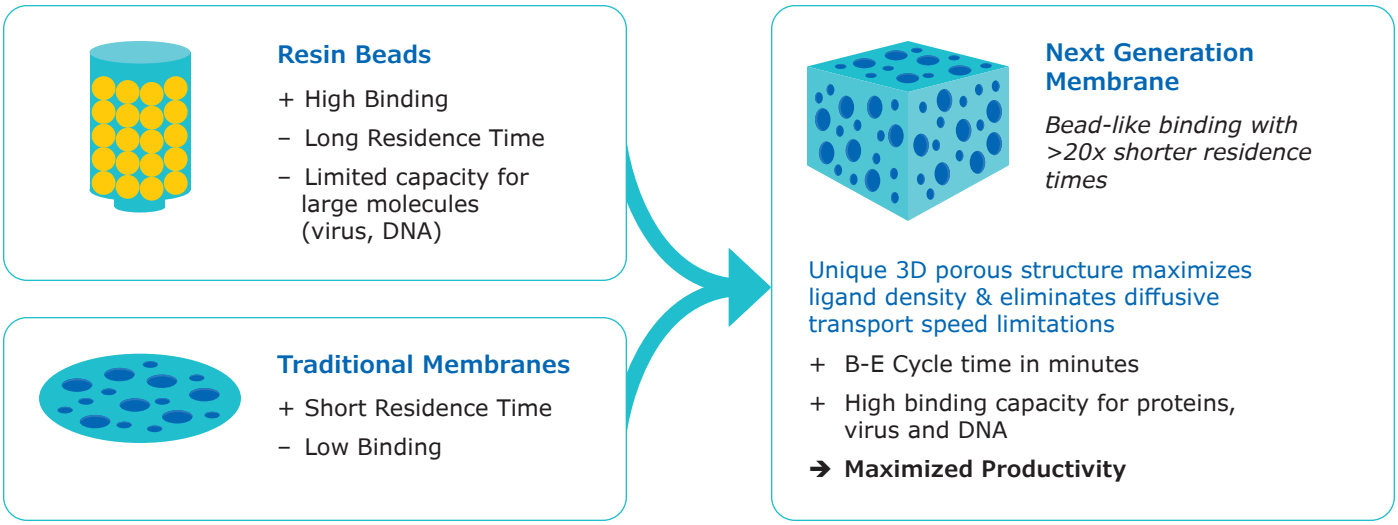
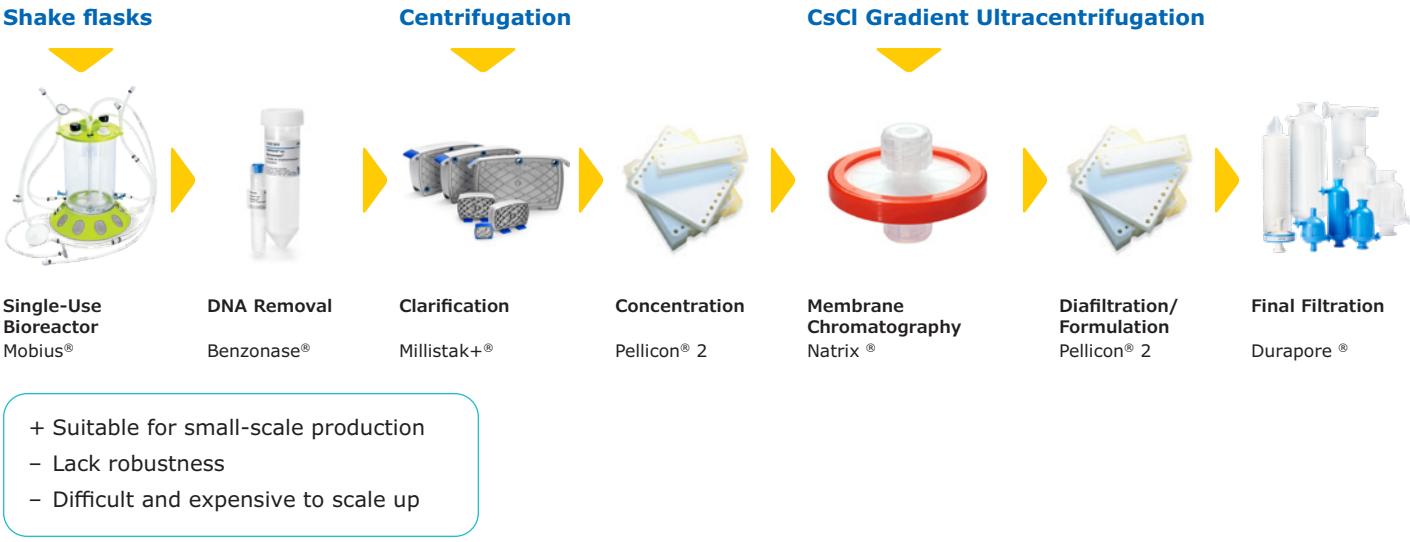


Figure 3. Natrix® chromatography membrane combines the high binding of resin beads and short residence time of conventional membrane adsorbers.



+ Suitable for small-scale production
– Lack robustness
– Difficult and expensive to scale up

Figure 4. Replacement of steps in the small-scale process enabled transition to a cost-effective, scalable template.

Load (VP/mL-media)	Recovery	HCP Reduction
6 x 10^{13}	76%	85%

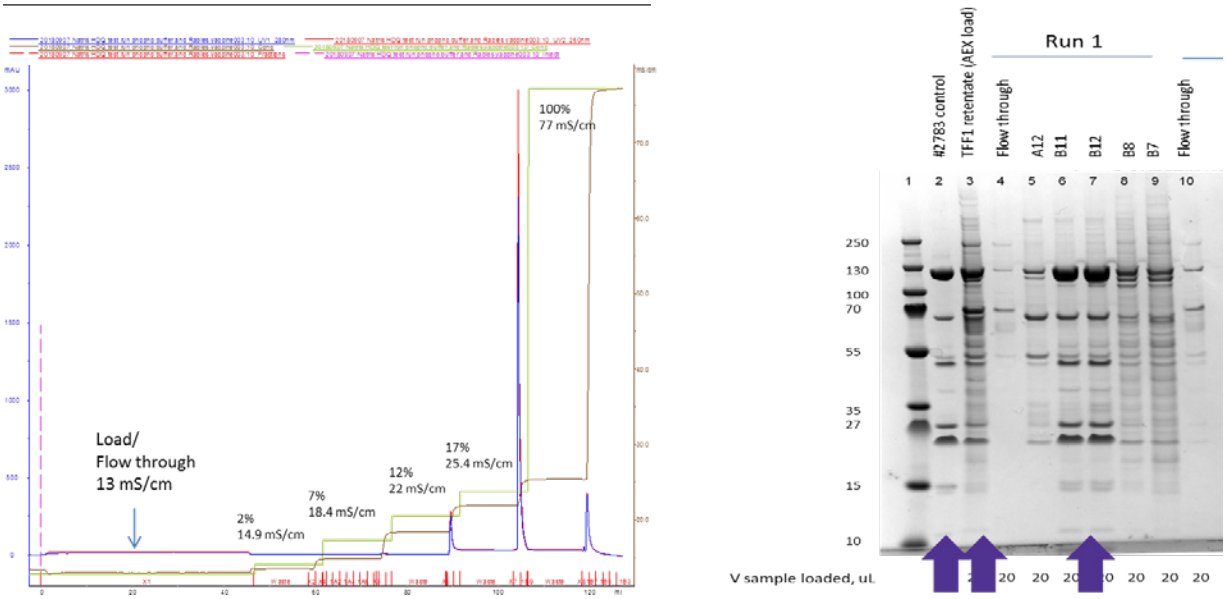


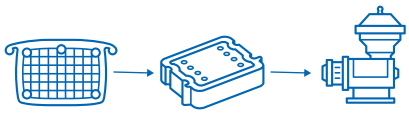
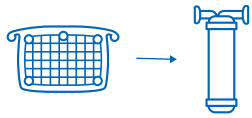
Figure 5. Use of the Natrix® Q chromatography membrane enabled good recovery of the virus and a significant reduction in HCP.

Balancing Productivity, Scalability and Purity

The limitations associated with conventional technologies such as centrifugation and ultracentrifugation are further magnified in the production of gene and oncolytic viral therapies. Compared to vaccines, these novel therapies require a very high quantity of virus and with intravenous administration, purity requirements are quite strict. The following case study demonstrates how replacement of centrifugation and ultracentrifugation steps enable a high-productivity, scalable process for production of an oncolytic viral therapy without compromising purity. This work was done in collaboration with the University of Guelph with funding from NSERC, the Natural Sciences and Engineering Research Council of Canada.

Newcastle disease virus (NDV) is an avian, enveloped single-stranded RNA virus with potent antineoplastic properties. High-titer NDV is typically produced from pathogen-free embryonated chicken eggs and processed using depth filtration, TFF and sucrose-gradient ultracentrifugation. Lab-scale processing requires nine hours, with two additional days for dialysis and polyethylene glycol concentration to remove excess sucrose which can be toxic if delivered intravenously. This process suffers from low productivity and throughput and would be difficult and expensive to scale.

For transition to a scalable process with improved productivity and overall economics, the TFF and ultracentrifugation steps were replaced with a single step of Natrix® Q anion exchange chromatography membrane. This intensification strategy allowed the entire purification process to be completed

	Traditional Process	Next Generation Process
Purification Process		
	Depth filter → TFF → Ultracentrifugation (UC)	Depth Filter → AEX (Natrix® Q)
Process Duration	9 hours*	0.5 hours
Product Recovery	65-70%	>90%
Scalability	✘ UC difficult and expensive to scale up	✓ Depth filter and AEX easily scalable

* An additional 2 day dialysis and PEG concentration is required to remove concentrated sucrose from UC

Figure 6. Comparison of traditional and next generation processes using membrane chromatography for production of an oncolytic virus.

in thirty minutes, with greater than 90 percent recovery, compared to 65-70 percent recovery with the original process (Figure 6). This increase in recovery not only improved downstream throughput and economics, it enabled downsizing of upstream production, specifically the number of eggs required, by sevenfold.

Purity was not sacrificed to achieve improved productivity and scalability. Figure 7 is a reconstructed chromatogram based on an offline analysis of virus titer and host cell protein (HCP) concentration.

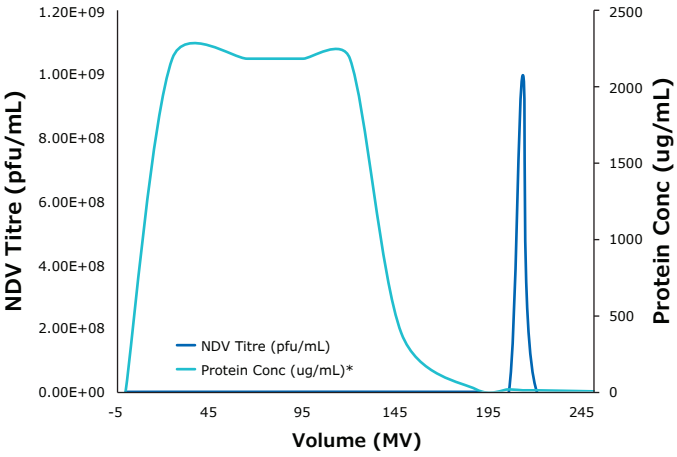


Figure 7. NDV was concentrated by 13-fold compared to the starting feed and 3.2 log reduction of HCP was achieved.

With optimized loading conditions, a majority of the HCP flowed through the membrane, while the viruses were captured. Upon elution, NDV was concentrated by 13-fold compared to the starting feed and 3.2 log reduction HCP was achieved.

The large size and pleomorphic nature of this virus require good accessibility to functional groups in the chromatography media for an acceptable binding capacity. This is key advantage of membranes over resins, as the small pores at the resin bead surface create a size-exclusion effect and prevent large viruses from diffusing into the pores and binding to the interior functional groups. Moreover, the slow diffusive mass transfer required with resin chromatography leads to long residence time , which significantly decreases productivity. For this NDV process, a resin-based purification would require a five-hour loading compared to ten minutes for the membrane-based process.

Large-scale production of clinical-grade material was enabled with this intensified process. This approach can also help drive down costs by streamlining production processes and reducing the capital and operational cost required for a commercial-scale process and facility. An important insight is that for viruses produced in cell culture, downsizing of upstream processes enabled by increased downstream recovery, can facilitate conversion from a stainless steel bioreactor to single-use, leading to significant capital and operational savings. This example is an important reminder to think holistically when considering the economic benefits of intensified process; advancements in a single unit operation can unlock many opportunities in the overall production process.

Improving the Affordability of Vaccines

This case study describes how process intensification can drive down the cost of manufacturing inactivated viruses by reducing the manufacturing footprint. The reduction in cost of goods (COGS) can then translate into more affordable poliovirus vaccines for low- and middle-income countries. This project was a collaboration with Univercells and Batavia Biosciences, funded by Bill & Melinda Gates Foundation, in support of the World Health Organization's (WHO) Global Polio Eradication Initiative.

Development goals were threefold: increase upstream productivity with a single-use fixed-bed bioreactor; use Natrix® Sb chromatography membrane for single-step purification; and incorporate these technologies into an automated, continuous-production platform with a small footprint and low cost.

Cultivation of virus cells was carried out in the fixed-bed bioreactor which achieved a high cell density; the harvest was then sent for inline downstream processing and inactivation (Figure 8).

Natrix® Sb, a cation-exchange membrane with HIC modality was used for single-step purification of the virus (Figure 8). Similar throughput, recovery and purity were observed for all three sIPV serotypes; only sIPV-3 data are shown. Clarified sIPV feed was loaded to more than 81,000 D-antigen units per mL membrane. At elution, the material was concentrated 35-fold with very good recovery and a purity profile meeting WHO's requirements for HCP and DNA. With this high level of load and recovery, one cycle from the scale-down device generated sufficient sIPV-3 material for more than 9,000 doses of vaccine. With a 500 mL device for large scale manufacturing, we extrapolated that one cycle can supply 1.2 million doses of sIPV-3 material.

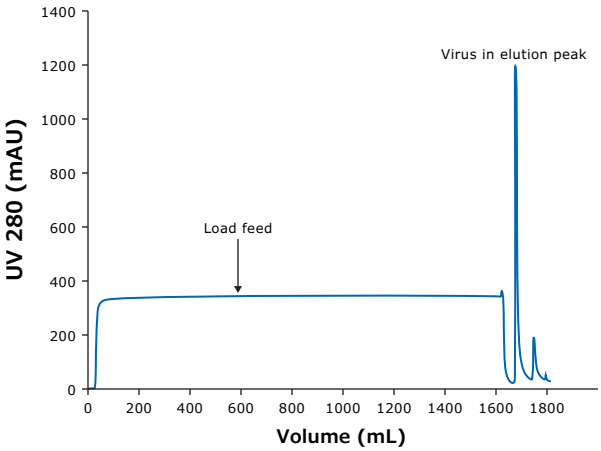


Figure 8. Single-step purification with Natrix® Sb chromatography membrane.

Use of high-productivity upstream and downstream technologies enabled the entire production process to be self-contained into a series of six square-meter isolators, that can be placed in BSL-3 pod. The pod-based facility is compliant with new polio virus containment requirements outlined in WHO's Global Action Plan. This process intensification and resulting reduction in operation costs demonstrate that with less than a $25 million facility investment, it is possible to supply 40 million doses of trivalent sIPV vaccine annually, at less than 30 cents per dose, a fivefold reduction compared to current costs.

The miniaturized, modular facility enabled by process intensification also creates the foundation for a new scaling strategy. Instead of the traditional approach of scaling the size of process units and enlarging the overall facility, increased production can be enabled by adding identical, small-footprint processes and/or facilities. With this scale-out, rather than scale-up, approach, technical and regulatory scale-up activities are not required, as there is no change to the process. Risks are mitigated at an early phase by reducing the investment and accelerating development efforts for both the process and facility. The scale-out approach also allows more localized manufacturing strategy, where many identical facilities, each with a small footprint can support in-country, for-country production.

Envisioning a Platformable Purification Strategy

For viruses that are expressed in complex systems, purification processes require multiple steps to achieve the target purity including precipitation, centrifugation, ultracentrifugation, size exclusion and ion exchange chromatography. Adding to this complexity is the diversity of viruses and upstream production methods; in many cases, a unique purification process is required for every virus type and possibly every strain. For these applications, a more powerful separation technique is required to streamline the process without compromising critical quality attributes.

As a result of its highly specific interactions between target molecules and immobilized ligands, affinity chromatography is one of the most powerful separation techniques. When formatted as single-use membranes, it has the potential to be used as a platform chromatography technology for high-throughput, cost effective virus purification. Each virus could be purified with a different affinity media, followed by one or two polishing steps; using this approach, the overall downstream process and facility would require little to no modification. When coupled with high-productivity, single-use technology, the cost savings and flexibility can be further improved.

To explore the potential of single-use affinity membranes in virus production, a proof-of-concept study was conducted in collaboration with the University of Guelph. In the study, a commercially available ligand was coupled to the activated base membrane. Influenza virus produced in cell culture was loaded onto the membrane at $2x10^6$ HA units per ml-membrane. At elution, 95 percent recovery was achieved, with more than 99 percent removal of HCP and a 30-fold virus concentration (Figure 9).

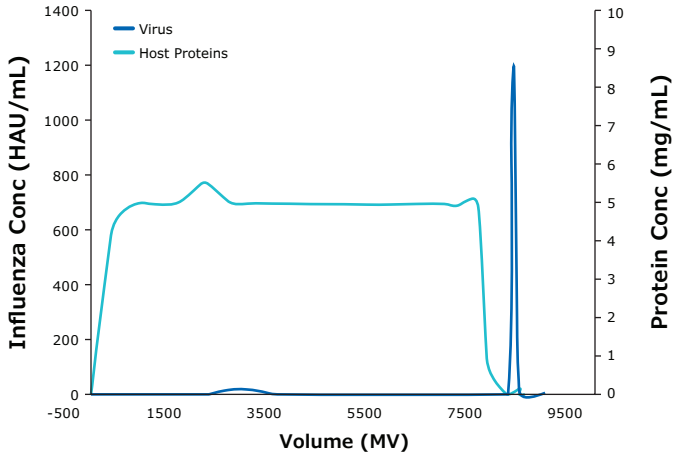


Figure 9. Purification of influenza virus using affinity membrane chromatography delivered a 30-fold concentration and 99.9% HCP clearance (3LRV).

Extrapolating these results, a 500 ml affinity membrane device could process allantoic fluid harvested from one million eggs a single eight-hour shift. As compared to affinity resin, productivity can be significantly improved due to the significantly reduced cycle time which results from the reduced residence time.

Compared to multistep purification processes reported in the literature for egg-based influenza virus, the process described above is simplified to clarification and affinity chromatography, with the material meeting the necessary purity requirements for further processing (Figure 10).

Creating a new Paradigm for Virus Production

Many virus purification processes require long development timelines, significant facility and equipment costs and are limited in terms of their flexibility. Process intensification strategies, such integration of single-use membrane chromatography, offer the potential for simpler, less expensive processes and more efficient production, from lab to commercial scale.

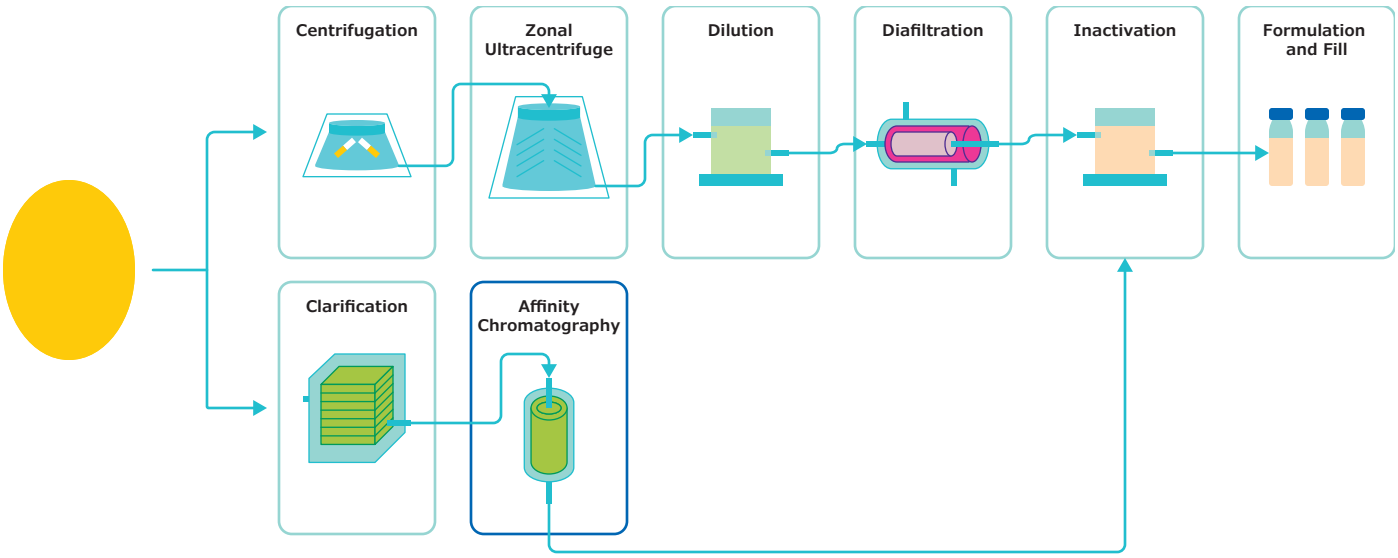


Figure 10. Use of affinity membrane chromatography has the potential to significantly reduce the production of egg-based influenza vaccines. Process Reference: Kon, Theone C et al. "Influenza Vaccine Manufacturing: Effect of Inactivation, Splitting and Site of Manufacturing. Comparison of Influenza Vaccine Production Processes." PLoS One vol. 11,3 e0150700. 9 Mar. 2016, doi:10.1371/journal.pone.0150700

With an intensified workflow, process development can be accelerated, and construction lead times significantly shortened, as a result of the ability to build smaller facilities with reduced hardware and storage requirements.

Adoption of intensified processes, especially in combination with single-use technologies, can reduce costs by eliminating the need to invest in traditional, expensive production facilities. Large expenditures can be delayed beyond the early clinical phase, when risk is highest and variable costs can be distributed over the drug's lifecycle. Streamlined process and right-sized unit operations further reduce capital and operational costs.

Finally, intensified processes improve facility utilization. Greater flexibility enables more rapid product changeover; this translates into more batches produced annually and enables use of multi-product facilities that can more rapidly to varying market demand or emerging threats. If there is a need to reconfigure the facility for new processes to support emerging, novel therapies, the time required for this can be significantly shortened.

Given the needed central role of viruses in many therapeutic modalities, the ability to accelerate process development and reduce the cost of manufacturing will undoubtedly have a positive impact on the global population.

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