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Dear Colleague,

Pathogens pose massive health and economic risks. These and other Infectious diseases emerge or reemerge on a regular basis, threatening populations around the world from our densely populated urban centers to the most rural locations.

The constant threat posed by these pathogens drives demand for effective, safe and affordable vaccines. Unfortunately, the complexity and variety of vaccine types and manufacturing methods have prevented establishment of robust processing templates, which can leave vaccine manufacturers on their own to develop customized approaches for each pathogen and each outbreak.

At MilliporeSigma, our mission is to “solve the toughest problems in life science”. One of these problems is the need for more efficient and cost-effective manufacture of vaccines. To this end, we actively collaborate with academia, vaccine researchers and manufacturers with deep knowledge of their vaccine candidate and clinical aspects to develop and optimize innovative tools, processes and strategies to resolve bottlenecks and accelerate the availability of desperately needed vaccines.

In the following pages, you will find a series of case studies highlighting our recent collaborations with organizations and thought leaders on the front lines of the battle against these challenging pathogens. I know you will be inspired by these initiatives and I encourage you to contact us to explore new ways to tackle this problem, together.

Sincerely,

Darren Verlenden

Senior Vice President
BioProcessing, Process Solutions
MilliporeSigma

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Vaccine Manufacturing: Collaboration Helps to Overcome Vaccine Process Challenges

This white paper showcases collaborative technology-development efforts carried out by the DiViNe consortium to advance the use of an innovative affinity chromatography platform during vaccine manufacturing. This paper also discusses how long-term partnerships with strategic technology experts can help overcome inherent issues that arise as promising vaccine candidates move through scaleup, from proof-of-concept through commercial-scale manufacturing.

Authors

Achim Schwämmle, R&D Manager, Analytics & Application, Life Science Process Solutions, Bioprocessing and R&D Purification for MilliporeSigma, the Life Sciences company of Merck KGaA, Darmstadt, Germany.

Ranjeet Patil, Segment Head, Vaccine and Gene Therapy, Bioprocessing, Life Sciences Process Solutions for MilliporeSigma, Bedford, MA, USA.

Mikkel Nissum, Director, Lead Technical Development Leader, Technical R&D, GSK Vaccines S.r.l. (Siena, Italy)

Introduction

Vaccines are one of the most cost-effective health interventions available, and demand for safe and affordable vaccines continues to grow, to protect adults and children from numerous bacterial and viral pathogens. However, thanks to the sheer number and diversity of today's promising vaccine types, and many competing cell culture, production and purification options that are available, development of large-scale vaccine production process remains an inherently challenging undertaking. As a result, the developmental pathway for promising new vaccine candidates is far from assured.

The decisions related to unit operation selection and process design have significant technical and business implications for the success of any vaccine-manufacturing effort through each stage of development and scaleup. Technical decisions related to process development have direct business consequences, in terms of the overall technical and commercial viability of the proposed production route, the required capital, operating costs, critical timeline implications, final per-dose vaccine costs and more.

Vaccine developers should work to develop close partnership with key technology providers to leverage their broad and deep domain expertise and operating experience and to gain access to that partner's diverse cohort of internal experts (including not only biopharmaceutical scientists and engineers, but modeling and simulation experts, economists, regulatory-review experts and more). Such partnerships between vaccine manufacturers and technology providers can accelerate the development of purpose-built solutions and eliminate process bottlenecks by leveraging the core competencies and insights of each partner.

At issue: Enabling vaccine production in developing nations

In recent years, the vaccine-manufacturing paradigm has been shifting away from primary reliance on large, centralized vaccine-manufacturing facilities in North America and Europe, toward production that is carried out in smaller, decentralized and localized facilities all over the world. This newer approach is helping to greatly expand patient access to lifesaving vaccines. For instance, today, localized vaccine-manufacturing capabilities in developing nations now account for nearly half of all vaccines purchased by the United Nations agencies for use in the developing world — up from less than 10% in 1997.[1]

However, the ability to build and operate safe and reliable vaccine-production facilities in developing regions is often hampered by a range of endemic issues. These include non-existing or insufficient manufacturing and supply-chain infrastructure, inadequate cold-chain transportation and storage capabilities, the inability to stockpile supplies, insufficient clean water supplies, erratic power-grid reliability and more.[2] The ability to design robust vaccine-manufacturing platforms, based on more-streamlined, nimble designs and more sustainable operational concepts, remains an aspirational goal in the vaccine community to help to address these critical issues and enable safe and reliable localized production of vaccines in developing regions.

During vaccine manufacturing, downstream purification to capture and concentrate the vaccine antigen and to separate unwanted impurities and contaminants is an essential part of the process, yet it is inherently challenging and costly. The conventional purification process typically involves multiple, capital-intensive steps, involving project-specific combinations of such unit operations as chromatography, precipitation, ultracentrifugation, membrane filtration, tangential flow filtration, enzymatic digestion and more. By some industry estimates, 80% of the overall manufacturing costs associated with the production of vaccines and biologic therapies is related to efforts to remove contaminants and impurities.

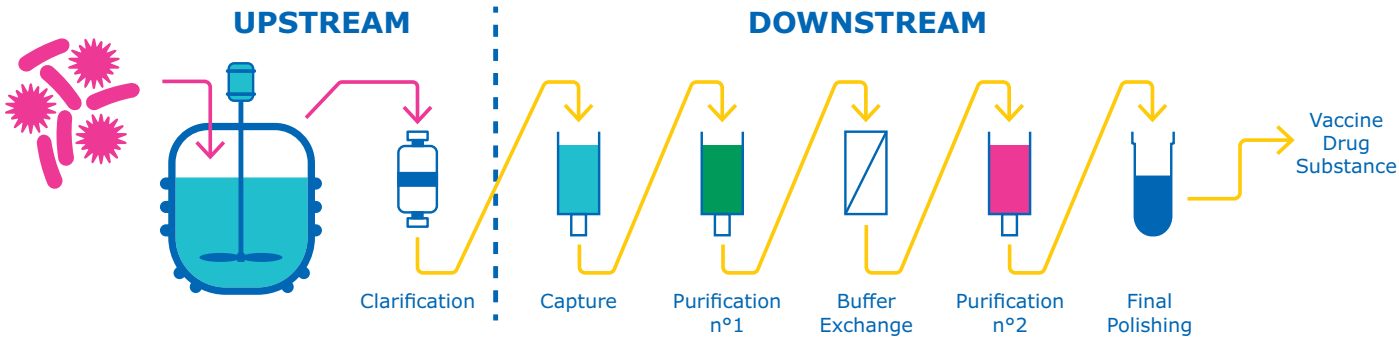


Figure 1. Current situation: multiple steps process = time and water consuming, expensive and limited yields

A key fact impacting the potential viability of the proposed system design is that any time a multi-step separation train is required for vaccine purification, the process will incur penalties in terms of product yield losses, longer batch cycle times, added capital expenditures, increased operating costs, costs added to meet energy and water/wastewater requirements (for rinsing and washing) and more. Thus, the ability to reduce the number of process steps through a streamlined design concept is highly desirable to make the vaccine process as viable and robust as possible.

Advancing vaccine purification through enhanced affinity chromatography

Today, six global companies are working together as part of the DiViNe Consortium (https://divineproject.eu), to address several key challenges that limit the effectiveness of the current downstream purification approaches that are widely used during vaccine manufacturing. The technology partners involved in this group are working to demonstrate and validate several critical technology breakthroughs that will establish a highly efficient vaccine-purification process.

At the heart of the robust, scalable vaccine-purification paradigm being pursued by the DiViNe Consortium is a state-of-the-art affinity chromatography step that uses engineered affinity ligands for improved performance. Specifically, the DiViNe member companies are using highly functionalized Nanofitin ligands to enable advanced vaccine purification during the production of a diverse variety of vaccine types. Affinity chromatography using Nanofitin ligands takes advantage of highly specific interactions between the target molecule (in this case, the vaccine antigen) and a ligand whose properties and characteristics have been tailored or engineered to maximize its affinity for that target molecule.

The affinity chromatography matrix is conjugated to immobilize the chosen affinity ligand. In the case of a packed-column affinity chromatography system, spherical beads of high-porosity polymeric resin are used as the chromatography matrix. Other types of affinity chromatography media (using, for instance, porous monolithic materials and membranes instead of polymeric resin beads) are also available, to meet the needs of the individual application.

As the feed stream flows through the affinity chromatography column, target molecules are separated from the flowing stream and are temporarily bound to the affinity ligands, while unwanted impurities and contaminants (such as culture media ingredients, cell membrane components, host cell proteins and nucleic acids) flow out of the column as an effluent stream. The target molecules are eventually released from the affinity ligands, using relatively mild elution conditions (so as not to damage the structure or viability of the manufactured vaccine product).

The DiViNe Consortium — Pioneering advances to improve purification during vaccine manufacturing

The DiViNe Consortium has enlisted six global companies to pool their diverse expertise in an effort to address several persistent challenges that arise during downstream purification, which is a critical aspect of any vaccine manufacturing effort. Through this five-year project, the partner companies are working together to address the following objectives:

- Set new performance goals for downstream purification
- Identify and rectify process inefficiencies that are inherent during downstream purification
- Improve recovery rates for target antigens

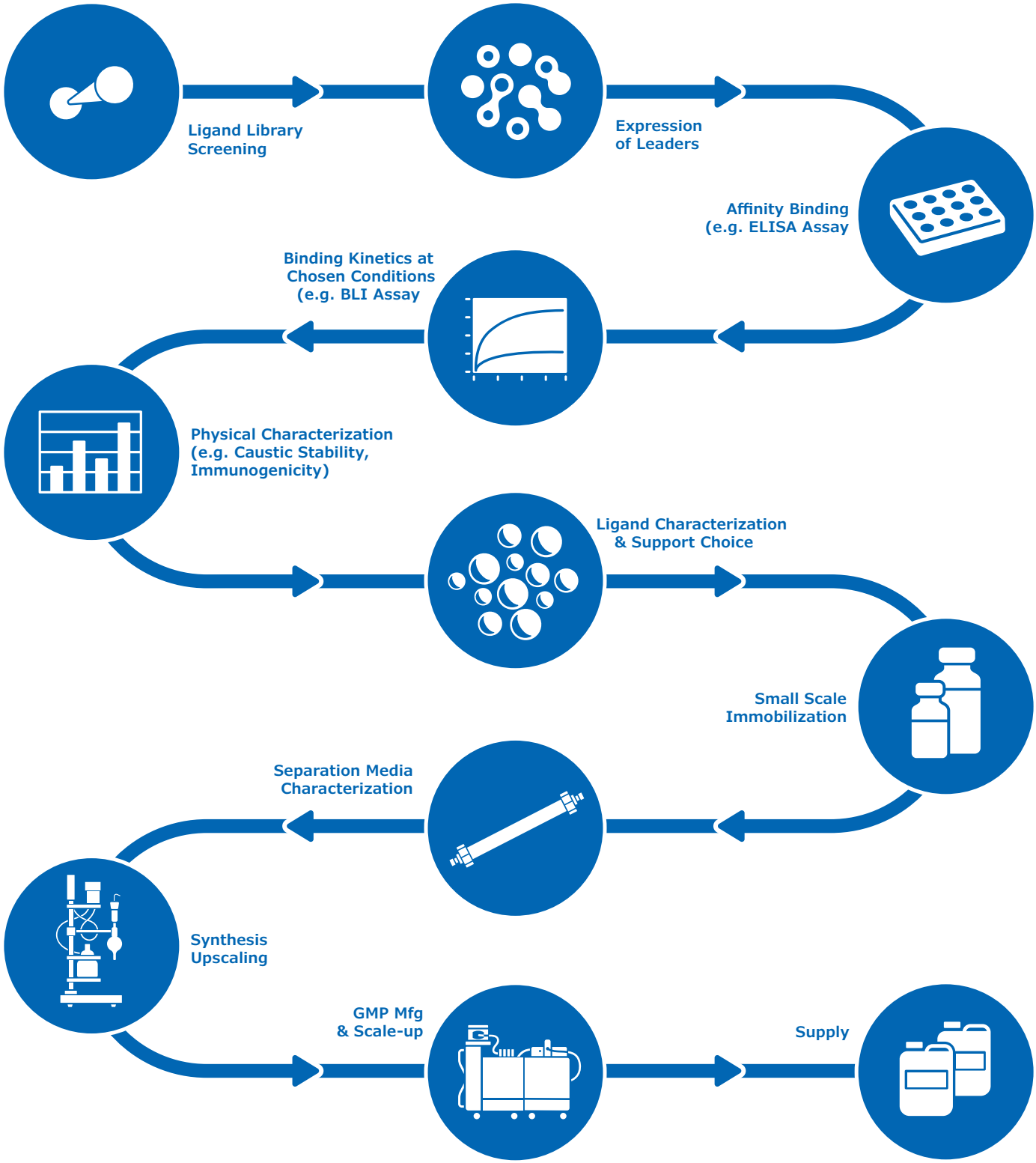
Collectively, these efforts are expected to enable state-of-the-art, robust vaccine-manufacturing systems that can make localized production opportunities more widely available — and thus lifesaving vaccines more affordable — than ever before.

Each of the six DiViNe Consortium partners brings its own key technology offerings and science and engineering expertise to bear on this collaborative effort:

- **GSK Vaccines S.r.l. (Siena, Italy) is providing several diverse vaccine candidates.** These are being tested using the innovative affinity chromatography designs and enhanced water-recovery method that are being advanced via the consortium effort. The final designs will be implemented to support industrial-scale process production
- **Merck KGaA, Darmstadt, Germany, and its Life Science company MilliporeSigma (Bedford, Mass.)** is providing affinity chromatography expertise, in terms of the production and functionalization of the required affinity chromatography material, the coupling technologies needed to attach the affinity ligands to the chromatography resins beads, and overall system design, engineering optimization and troubleshooting expertise
- **Affilogic (Nantes, France)** is developing engineered Nanofitin ligands
- **Aquaporin (Copenhagen, Denmark)** is providing proprietary, hollow-fiber Aquaporin Inside™ membranes to enable water reuse
- **GenIbet Biopharmaceuticals (Oeiras, Portugal)** will perform all required testing and be responsible for cGMP manufacturing compliance (taking into account quality and regulatory requirements), and identify a path to validate this global, compliant, environment-friendly process for vaccines manufacturing
- **Instituto de Biologia Experimental e Tecnologia (iBet; Oeiras, Portugal)** is functioning as Project Coordinator, and is responsible for process validation, including quality control and upstream and downstream integration as the final prototypes are scaled up.

The consortium is being advised by an external expert board, including members of the GSK Vaccines Institute for Global Health and the European Vaccine Initiative (EVI). The DiViNe Consortium has received funding from the European Commission's Horizon 2020 research and innovation program under Grand Agreement 635770.

Affinity chromatography has key performance advantages over many other forms of chromatography in that it can achieve extremely precise separations with relatively high capture efficiency and hence high yields. And importantly, when the right ligand can be engineered, the bulk of purification can often be achieved in a single step, thereby helping to streamline the overall system design through process intensification.



Validating affinity chromatography on diverse vaccine types

Today, the DiViNe Consortium is working to demonstrate and validate the use of affinity chromatography using highly functionalized ligands to improve the downstream purification on three diverse families of widely used antigens targets, in this order:

- **Family 1 — Carrier proteins for conjugated polysaccharide vaccines.** These typically experience relatively low yields using traditional purification options; The initial work is focusing on purifying the CRM197 carrier protein.
- **Family 2 — Protein antigens.** These are particularly hard to separate from byproducts such as truncated forms during manufacturing.
- **Family 3 — Fragile enveloped viruses.** These are not amenable to traditional purification systems.

Based on the initial demonstration-scale findings and additional process modeling, the DiViNe Consortium anticipates that this advanced approach to downstream purification (based on affinity chromatography instead of a multi-step chromatography process) will be able to dramatically improve the recovery yield of vaccine production — by as much as 3–5 times — while streamlining overall system design and complexity and reducing production costs.

The use of a semi-standardized platform approach — another key objective being pursued by the DiViNe Consortium — to carry out downstream purification via a single-step affinity chromatography system has the potential to give vaccine makers greater flexibility to change the product slate produced in the facility, or to respond relatively quickly to fluctuations in product demand, simply by modifying which functionalized ligand is used in the affinity chromatography step. This has the potential to usher in a new era in flexible, cost-effective vaccine manufacturing.

Process intensification delivers results

The ability to use affinity chromatography to intensify the purification of manufactured vaccine species while reducing the number of process steps required can help vaccine manufacturers to achieve a number of important objectives:

- Reduce the number of process steps and unit operations that are required to purify the product
- Reduce overall system complexity, by minimizing equipment requirements and associated capital, operational and maintenance costs
- Improve conversion yields, maximize production capacity, and reduce product losses and off-specification batches by switching to a one-step affinity chromatography approach
- Promote a simplified, platform- or template-design approach to improve manufacturing flexibility, minimize the facility size and footprint, and support technology transfer that can enable localized vaccine production
- Minimize the environmental impact of the purification train by reducing the number of process steps, thereby trimming energy use and water consumption and improving closed-loop water reuse
- Enable a flexible downstream purification paradigm that allows vaccine manufacturers to increase or decrease production capacity as needed, or handle different vaccine types, using only minor engineering or operating modifications
- Decrease time to market, which both increases patient access to lifesaving vaccines and allows vaccine makers to respond quickly in the event of fast-emerging epidemics and pandemics
- Allow for cost-effective, localized vaccine production for small markets, which may not otherwise be considered economically viable

While the initial goal of the DiViNe Consortium is to demonstrate improved purification of several specific vaccine types (as noted above), the breakthroughs being pursued by the group are also expected to have widespread applicability for manufacturers of many diverse types of vaccines, and for producers of other biopharmaceutical therapies, as well.

Targeting adventitious viruses using affinity chromatography

In addition to its use as an integral step during the purification of vaccine targets during manufacturing, ligand-based affinity chromatography also has the potential to preferentially remove unwanted or adventitious viruses that threaten the integrity of the final vaccine product.

Many of today's biologic therapies, vaccines and cell- or gene-therapy products are produced in animal- or human-derived cell lines, which increases the risk of adventitious viral contamination. Viral contamination not only results in lost product batches, but it engenders added equipment-sterilization costs and potential facility shutdowns, thereby incurring cost penalties on vaccine and biopharmaceutical producers that can run into the millions of dollars.

To reduce, remove or inactivate unwanted viral contamination from manufactured vaccine batches, manufacturers typically rely on three fundamental approaches:[3]

- Prevent the presence of the virus by careful source-material selection
- Detect the viral contaminants using in-process and lot release testing
- Remove the unwanted virus fragments using multiple unit operations during downstream purification

While current ion-exchange chromatography methods (using cation-exchange and anion-exchange chromatography) are often used for viral-vaccine and viral-vector purification, this approach typically exhibits relatively low capacity and poor selectivity for adventitious viruses. This opens the door for the use of affinity chromatography using tailored ligands instead, as this approach provides improved selectivity and optimized capture that is needed to remove adventitious viruses during the large-scale manufacture of vaccines.

Partnerships to enhance bioprocessing efforts: A new paradigm for process development

Given the immense complexity and risk associated with scaling up any promising vaccine discovery, it is more important than ever for vaccine manufacturers to work in close, collaborative partnership with their experienced technology providers. Such partners can provide critical advisory support throughout the entire effort, providing technical and business support from the conceptual design stage through scaleup and startup. The technology provider's input is especially important during the purchase, startup, commissioning and validation of critical equipment components and systems — drawing upon its own vast experience base developed from work with other clients in the vaccine space, and thus bringing best practices to bear.

With the rapid emergence of new infectious diseases and increasing demand for lifesaving vaccines, vaccine developers need to be nimble and move toward more streamlined and efficient production platforms. When vaccine developers create an ongoing, collaborative partnership with a technology provider, each party is able to leverage the strengths and expertise of the other. Collectively, the partners are able to identify optimal engineering and design solutions and resolve technical and economic bottlenecks more quickly and efficiently.

Vaccine developers bring to bear their holistic understanding of the underlying science for identifying and producing promising vaccine antigens, and have broad experience in critical quality attributes and regulatory expectations. But they often have limited ability to develop or optimize the various unit operations and analytical tools that are needed to produce safe and reliable commercial quantities of the target vaccine, and optimize the needed unit operations in the most effective way.

Technology providers bring rich insights related to competing technology options and analytical methods used during the scaleup of vaccine-manufacturing processes. And through their industry-wide experience, such companies are able to leverage earlier learnings and best practices to solve critical process challenges quickly and efficiently.

Importantly, strong collaboration with experienced technology partners — those that possess broad and deep vaccine-manufacturing expertise across a diverse portfolio of customers in the vaccine sector — allows individual vaccine developers to recognize and confront complex challenges that can undermine the vaccine program as early as possible in the process.

Closing thoughts

Ongoing advances in state-of-the-art technologies are helping to:

- Improve downstream purification and improve water recovery during vaccine manufacturing
- Address pressing challenges that are routinely faced by vaccine manufacturers today, as they work to scale up promising vaccine breakthroughs, and develop and validate viable commercial-scale production routes

The development of the streamlined, platform-based system design described here — based on affinity chromatography using highly functionalized ligands to improve downstream purification — has the potential to greatly improve recovery yields for several different families of high-purity vaccine components, and can effectively minimize contaminants and impurities that both threaten product quality and impose financial penalties on the operation. Collectively, these advances — made possible through strong, sustained collaboration between a vaccine developer and its technology partners — are helping to support expanded access to affordable vaccines across the globe.

Authors

Achim Schwämmle is R&D Manager, Analytics & Application, Life Science Process Solutions, Bioprocessing and R&D Purification for MilliporeSigma, Darmstadt, Germany. He holds a graduate degree in Chemistry and a PhD in Organic Chemistry from the University of Stuttgart (Stuttgart, Germany).

Ranjeet Patil is Segment Head, Vaccine and Gene Therapy, Bioprocessing, Life Sciences and Process Solutions for MilliporeSigma, Bedford, MA, USA. He holds a BS in Biotechnology Engineering from Shivaji University (Kolhapur, Maharashtra, India) and an MS in Biotechnology from Northeastern University (Boston, Mass.).

Mikkel Nissum is Director, Lead Technical Development Leader, Technical R&D for GSK Vaccines S.r.l. He holds a PhD in Chemistry and Biochemistry from the University of Southern Denmark.

References

1. https://divineproject.eu/impact
2. Suzanne Shelley, Moving the Needle in Pediatric and Adult Vaccines, Pharmaceutical Commerce, April 2018; http://pharmaceuticalcommerce.com/brand-marketing-communications/moving-the-needle-in-pediatric-and-adult-vaccines/
3. Ferreira, Ph.D., Meghaan M., Laying the Foundation for Viral Safety, which appears in the MilliporeSigma-sponsored publication entitled Viral Safety: Are You Doing Everything to Mitigate Your Risk, published in *GEN*, 2017.

Acknowledgement

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Producing Conjugate Polysaccharide (CPS) Vaccines — When to Consider Customized Ultrafiltration Membranes

Dr. Paul Beckett, Merck KGaA, Darmstadt, Germany
Ranjeet Patil, Merck KGaA, Darmstadt, Germany

The conjugation of polysaccharide antigens to the immunogenic carrier protein is a critical production step in the manufacture of CPS vaccines. This reaction rarely goes to completion, which therefore requires a separation of the free polysaccharides from the conjugated product. This separation is typically done by tangential flow filtration and it is challenging due to the small size difference between the product and the free polysaccharide contaminant.

Customized TFF (Tangential Flow Filtration) membranes with tight pore size specifications have been shown to be effective in these applications, allowing maximization of product yield and purity.

Vaccines save millions of lives every year and improve the quality of life for countless others, preventing potentially life-threatening diseases and preventing considerable additional healthcare costs. Today, due to the inherent variability of competing vaccine types, most vaccine-production platforms involve a certain degree of customization. This means that there are very few widely appropriate process templates and similar vaccine types can be produced by a very different set of unit operations

Conjugate polysaccharide (CPS) vaccines provide lifesaving protection against a variety of bacterial infections, including pneumonia, Haemophilus influenzae type b (Hib) and meningitis. Global demand for CPS vaccines is expected to continue growing, thanks to the increased efficacy of such vaccines, and the ongoing evolution and implementation of national immunization programs in developing nations. For instance, the World Health Organization (WHO) recommendations now include many conjugate polysaccharide vaccines on high priority vaccine lists.

The polysaccharide antigens (which induce the immune response) from the bacterial capsule are not very immunogenic in their native state therefore they are chemically conjugated to protein carriers, most commonly an inactivated toxoid such as diphtheria or tetanus. This increases the intensity and duration of the host immune response and reduces the need for additional adjuvants in the vaccine preparation.[1]

In the case of CPS vaccines, the multi-step downstream purification process is complicated by a variety of factors. The manufactured vaccine usually combines a large number of polysaccharide serotypes to provide immunity against several bacterial strains. These serotypes are defined by specific polysaccharide chains, which vary greatly in both length and the complexity of their branching structure. This greatly increases the difficulty in platforming these processes – in essence a 12 serotype CPS vaccine is 12 distinct and separate processes/products that are then pooled to create the final formulation.

Developing cost-effective production routes

Conjugate polysaccharide vaccines are used worldwide, often in children and for the developing world. This produces great pressure on unit costs, $1-$5 per dose being a typical benchmark.

Manufacturers therefore operate under severe economic constraints and need to reduce production costs, preferably by designing and operating effective, flexible and robust processes that still manage to hit specifications. Pressure is also felt on vaccine development because of emerging threats and those vaccines that need to be made locally, where development needs to be fast, effective and still not cost more than a developing country can afford.

To ensure access to the most state-of-the-art technology options and best practices for process design, troubleshooting, process optimization and process intensification, vaccine manufacturers should work closely with strategic technology partners. Through close collaboration, these technology partners can help the manufacturer to design the most appropriate vaccine-production system — one that optimizes the performance of the essential (and often competing) technical and business objectives. These include:

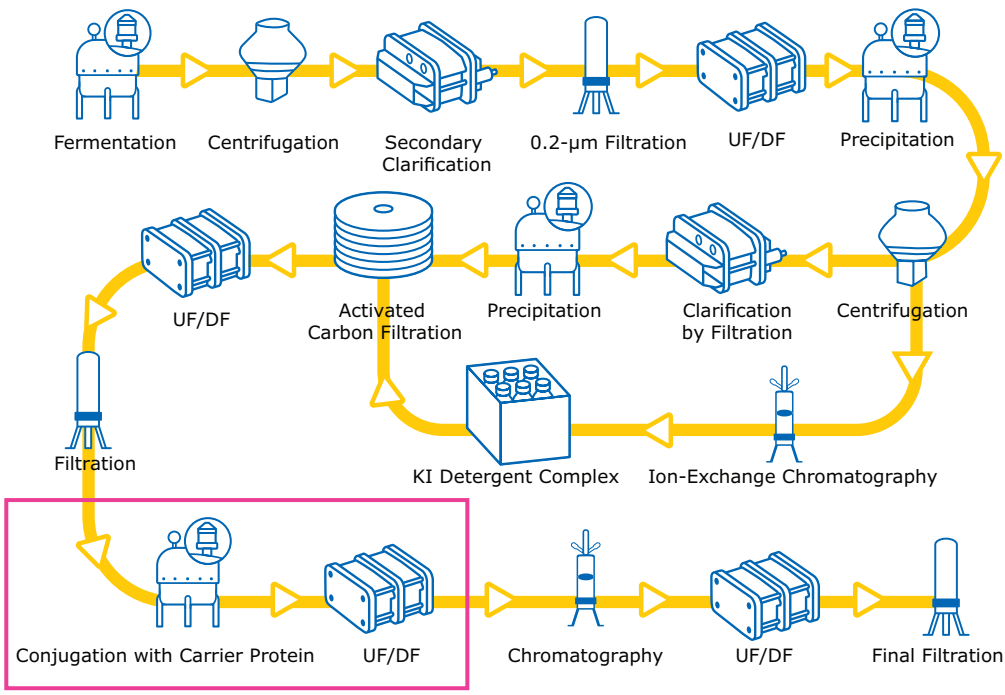
- Process efficiency
- Purity
- Product recovery
- Yield
- Speed to market
- Manufacturing flexibility
- Product safety
- Cost containment

Tangential flow filtration in CPS downstream processing

Downstream purification of CPS vaccines is typically carried out through the use of sequential filtration and chromatography steps, like the majority of biopharmaceutical processes. (Figure 1)

When it comes to the downstream purification of complex vaccine molecules with closely related contaminants, the yield versus the purity will always be a compromise. Robust process development allows for a window of operation of satisfactory values that consider such factors as feed stream consistency and the specification of membrane technology. If this window of operation is not broad enough the process loses robustness and out of specification results will become unacceptably frequent.

Conjugated polysaccharide vaccine manufacturing



Challenges:

- No established template exists for a vaccine purification platform due to complexity and diversity
- Carrier protein manufacturing process not shown
- Membrane pore size ratings are not the same from different vendors
- High number of diavolumes to remove residual linker and byproducts can adversely affect the yield
- Size of the conjugate is not much larger than the starting polysaccharide

During the conjugation process, the reactions carried out to chemically link the polysaccharide molecules to the protein carrier typically do not occur at a 1:1 stoichiometric ratio. As a result, the process stream will contain not only the desired conjugate polysaccharide molecules but also the residual phases of both unreacted protein and unconjugated polysaccharide. These unwanted species must be effectively and reliably removed during downstream purification, usually by TFF. For a variety of reasons this separation step creates particular challenges for manufacturers of CPS vaccines.

When it comes to CPS vaccines, several factors make it hard for semi-permeable TFF membranes to adequately and reliably separate the mixed species.

Specifically:

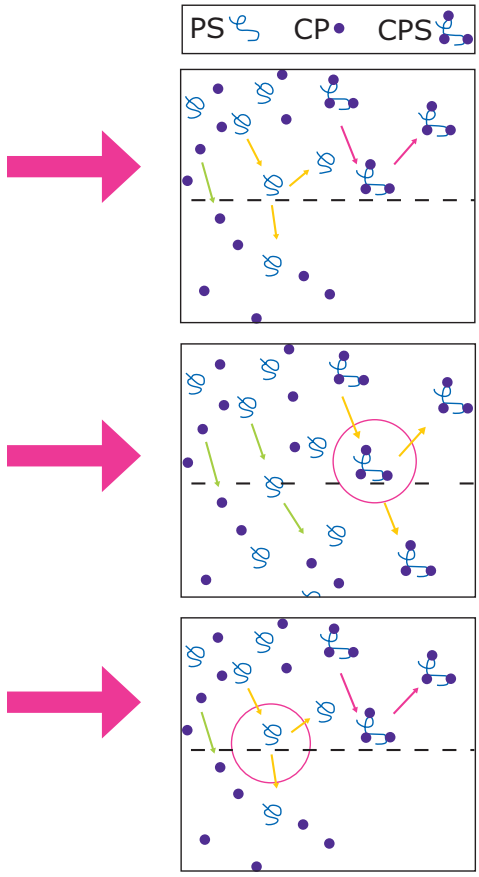
- There is often a relatively small size differential between the conjugate polysaccharide product (which is typically on the order of 500 kDa) and the unreacted free polysaccharide byproduct molecules (which are typically on the order of 300–500 kDa) and unreacted carrier protein. The actual difference in size varies greatly dependent on serotype.
- TFF operations are defined by having multiple passes through the feed channel during the process, up to and over 100. This means that small changes in nominal retention can have a disproportionate impact on final purity and yields. It is therefore desirable to have a size difference of at least 3x between the molecule you are trying to retain and that you are trying to separate. Such large differential in size is unusual in CPS vaccine purification.
- The innate variability of any semi-permeable membrane structure becomes more pronounced at larger pore sizes

Limitations of existing TFF membranes

An ideal pore size allows acceptable passage of unreacted polysaccharide (PS) and carrier protein (CP) with high retention of the conjugate polysaccharide (CPS).

Larger pores allow higher passage of the conjugate, and reduces the yield.

Smaller pores increase yield, but also increase the impurity level.



Membrane selection for TFF

This complex yet essential separation step can be improved through the use of process engineering upgrades to an existing TFF system and, where appropriate, the use of customized TFF membranes that can optimize the separation of species that have challenging size differentials. Success in these efforts can help manufacturers of CPS vaccines to improve both overall yield and purity in a reproducible fashion.

TFF membranes tend to be commercially available in a limited set of nominal molecular weight cut offs (NMWCO), such as 100kDa and 300kDa modified polyethersulfone or regenerated cellulose that are commonly utilized in CPS purification processes.

The limited availability of membranes with the precise pore size that is needed for a given CPS manufacturing process often leads vaccine manufacturers to make tradeoffs between yield and purity, rather than allowing them to optimize both for any given production run. As many vaccine manufacturers are already aware any operating decisions that are made to favor one objective at the expense of another (for instance yield-vs-purity) can have tremendous impact on both the reliability of the vaccine supply and profit margin.

As the conjugated polysaccharide molecule is often not much larger than the residual unconjugated polysaccharide from which it must be separated during the purification step, for some CPS vaccine products it may be difficult for standard TFF membranes to ensure a sharp and reliable separation.

When the membrane pores are too large, higher amounts of the product are allowed to pass through, reducing the overall yield of the process. Although this does not impact product quality directly, it does impact supply, which is typically critical for vaccines.

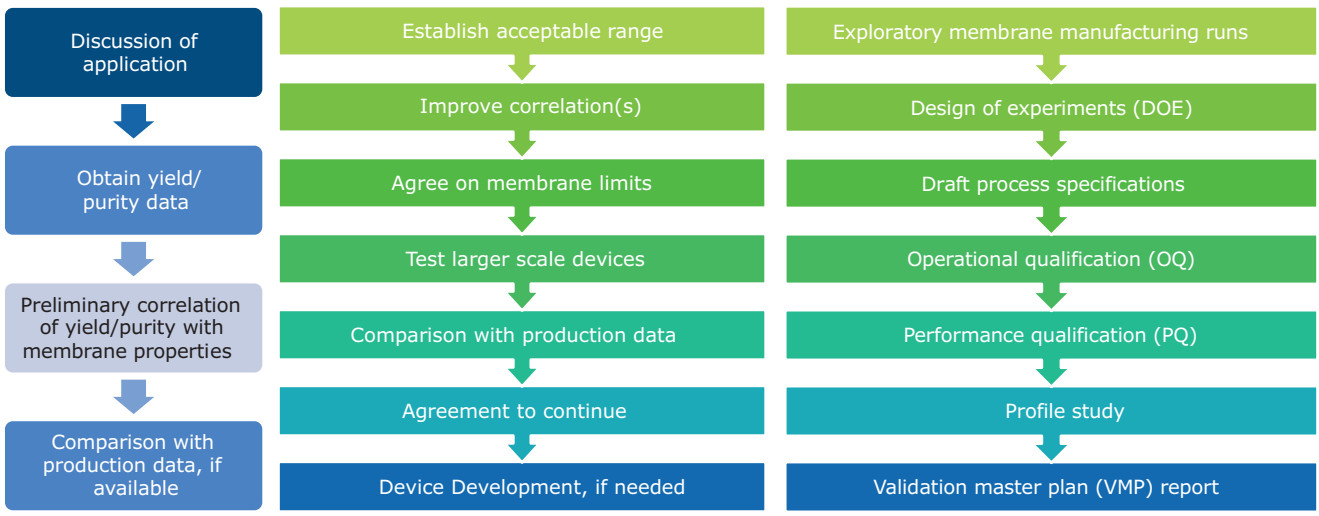
If the membrane pores are too small, yields of the conjugated product will be high. However, this will be counterbalanced by an associated rise in free polysaccharide, a product related contaminant that has to be kept to a minimum and typically has very tight specifications.

Due to this highly specific requirement for such a high-performance separation, a customized membrane may be required to provide a robust process that delivers high yield with low contaminant profiles. It is possible today to design a membrane with optimized pore size distribution and other favorable structural attributes.

Exploring the use of customized TFF membranes

By working in close collaboration with a technology partner — who may bring deep expertise in membrane design — CPS vaccine manufacturers are able to both troubleshoot and optimize their existing production processes, and, where appropriate, explore the concept of pursuing a customized TFF membrane solution. This option can help manufacturers improve the separation and retention of target molecules during the manufacture of CPS vaccines — especially when the different species in the mixed stream have great variability and very small differentials in their molecular weights or diameters.

Custom membrane development



The ability to fine-tune the physical attributes and performance characteristics of the TFF membrane, to suit the exact needs of the specific CPS vaccine product, allows the vaccine manufacturer to more reliably meet its purification challenges. To explore this option, the vaccine developer works in close collaboration with its technology partner to first determine the feasibility of the proposed customized membrane design, and then to develop key parameters.

During the feasibility study, a complex data set must be established — preferably using existing yield and purity data related to the vaccine manufacturer's existing TFF filtration process. The goal is to correlate existing yield and purity data to the existing TFF membranes that have been used to date, in order to identify performance shortcomings and to identify an ideal range of performance specifications for all of these key performance objectives.

If such a feasibility study indicates that a customized TFF membrane would be able to outperform the standard TFF membrane used to date, then the project advances to development phase. During the development process, the team works to:

- Establish acceptable performance and specification ranges for purity and yield
- Improve correlations between pore size and process performance
- Identify the range of pore sizes that would be optimal to meet the manufacturer's specifications for yield and impurity clearance
- Agree on membrane specification limits
- Test larger-scale devices (to identify membrane phenomena that often impact performance only at larger scale)
- Compare modeled results for the custom TFF membrane with actual production data from the vaccine manufacturer's existing system, to validate improved performance
- Develop a contractual agreement to continue
- Produce the custom membrane and develop the most appropriate TFF device

Next, working closely with the CPS vaccine manufacturer, the technology partner carries out exploratory manufacturing runs to produce samples of the custom TFF membrane, which are then validated and qualified. Such a process typically involves the following steps:

- Design of experiments (DOE)
- Draft of process specifications
- Operational qualification (OQ)
- Performance qualification (PQ)
- Profile study
- Validation and qualification of the final custom membrane composition and manufacturing process, and device design, to ensure consistent, reliable manufacture to meet the vaccine makers specification every time

This is a detailed project that takes considerable resources. However, if correctly designed and executed, can provide substantial improvements in both process performance and robustness for future manufacturing.

CONCLUSION

Conjugate polysaccharide vaccines are very challenging to process, requiring substantial compromises between purity and yields. This is particularly apparent during the separation of conjugated polysaccharide from free polysaccharide, which can be similar in molecular size. A combination of process parameter/system modifications and implementation of customized TFF membrane can be highly effective at solving these process challenges, if developed with an appropriate industrial partner.

Customizing the TFF membrane is effective at optimizing CPS and free polysaccharide separation. However, there are also some appropriate process changes that can be made with the existing membrane solution.

During TFF with large molecules in the feed stream a thin yet concentrated polarization layer (also called a gel layer) forms. This is not a caking layer but a specific phenomenon, and this layer has a great impact on both process performance and membrane retention.

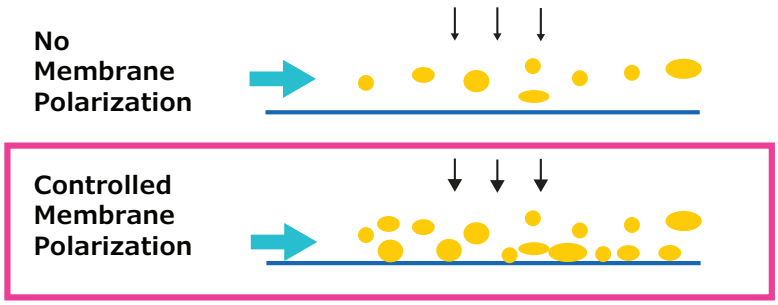
This polarization layer phenomenon is not a fouling layer, because it dissipates once the pressure in the system is reduced. Polysaccharides tend to display unusual polarization behavior, so managing the polarization layer appropriately is an essential part of successful TFF system operation.[1] If a polarization layer is uncontrolled, such as when the cross flow is too low or the transmembrane pressure (TMP) is too high, it can increase the apparent retention of the membrane. This is compounded by the unusual polarization behavior polysaccharides exhibit.

Open UF membranes, those of 100 kDa and above, typically have a low optimum TMP. This optimum can be so low that the base TMP, with the retentate valve fully open, exceeds this value and will over-polarize the membrane. However, this can be permeate flux controlled by use of a permeate pump, which regulates the TMP to the low values required. By regulating the polarization layer in this way retention can be kept within set limits and better control the separation of free polysaccharide and conjugated product.

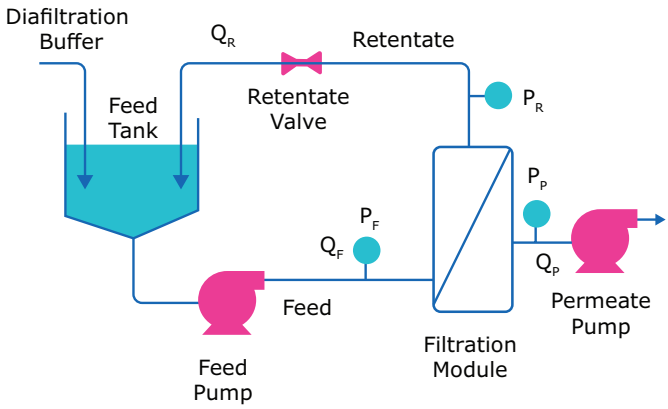
Reference

1. Analyses of polysaccharide fouling mechanisms during crossflow membrane filtration S. Nataraj a, R. Schom¨acker b, M. Kraumec, I.M. Mishra a, A. Drewsc

Importance of permeate flux control



- Open membranes can have very high conversions
- The optimum TMP can be very low and be uncontrollable with the retentate valve only
- Permeate flux pumps allow the TMP to be controlled
- Too high flux = too much polarization = too high retention



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

Jenner Institute, Oxford University, UK:
Sofiya Fedosyuk, Thomas Merritt, Marco Polo Perata-Alvarez, Phillip Angel Manning, Adam J Richie and Alexander D Douglas

MilliporeSigma, the Life Science business of Merck KGaA, Darmstadt, Germany:
Ada Lam, Nicolas Laroudie, Anilkumar Kangokar, Josselyn Haas, Anders Jonsson, Alex Xenopoulos, Youness Cherradi and Anissa Boumlic

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 use 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 (0.2 µm) 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.

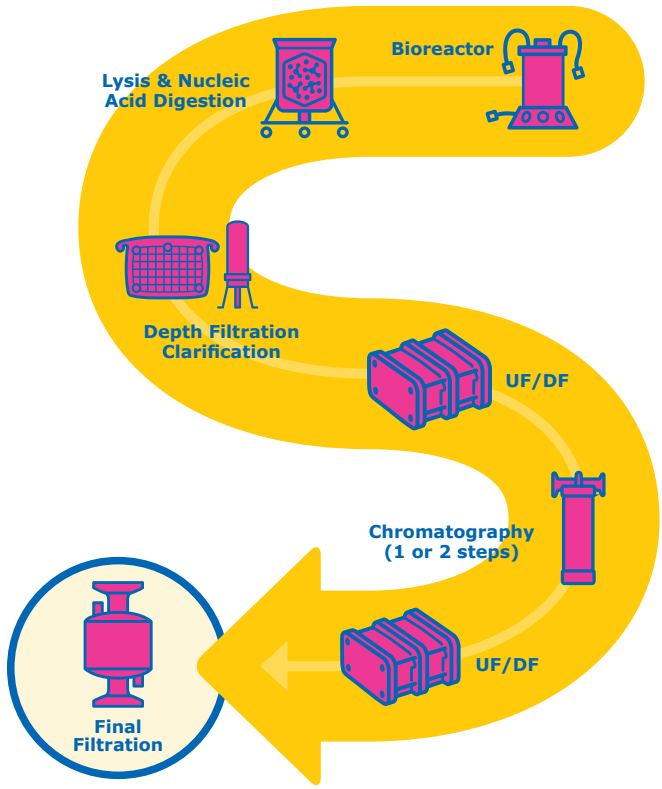


Figure 1. Schematic of a typical adenovirus vector manufacturing process.

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® Bioreactors	HEK293 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® nuclease 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.

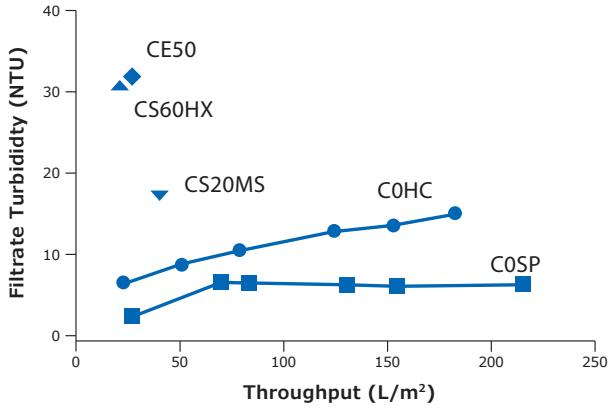
Downstream

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.

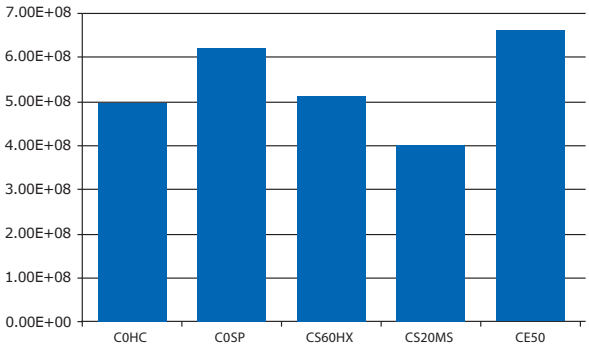
While adenoviral vectors are neutral, we still wanted to test different filters and select the best option to maximize adenovirus recovery. Table 2 describes the three Millistak+® depth filters and two Clarisolve® depth filters that were tested; each were different grades, micron ratings and charge.

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.



A. ChAdOx2-RabG – Titers after primary clarification VP/mL (Small scale trials)



B. Virus titers after clarification with Millistak+® HC Pro C0SP (VP/mL) (2-4 L scale)

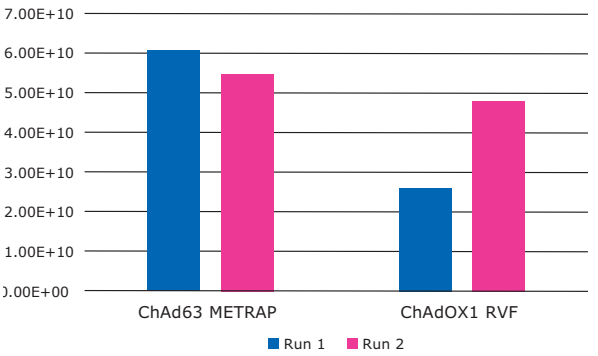


Figure 3. Evaluation of virus recovery using different depth filters. The Millistak+® HC Pro COSP performed best in terms of turbidity reduction and virus recovery.

Figure 3 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 C0SP 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® XL 150 Millipore Express® 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 (0.1m²) 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 Natrix® Q chromatography 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 exchange 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 Natrix® Q chromatography membrane 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 5 & 6). 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).

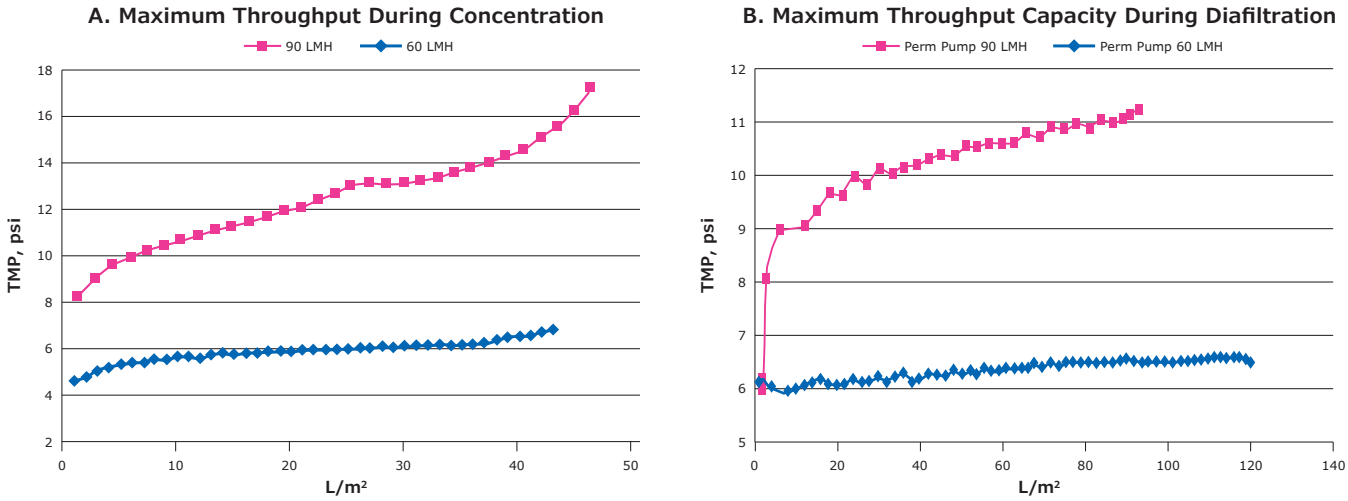


Figure 4: Small scale trials of the Tangential flow filtration experiment with clarified lysate of ChadOx2-RabG using 300 kDa MWCO Biomax® PES C-screen Pellicon® 2 Mini cassette. A. TMP excursion during concentration: B. TMP excursion during diafiltration.

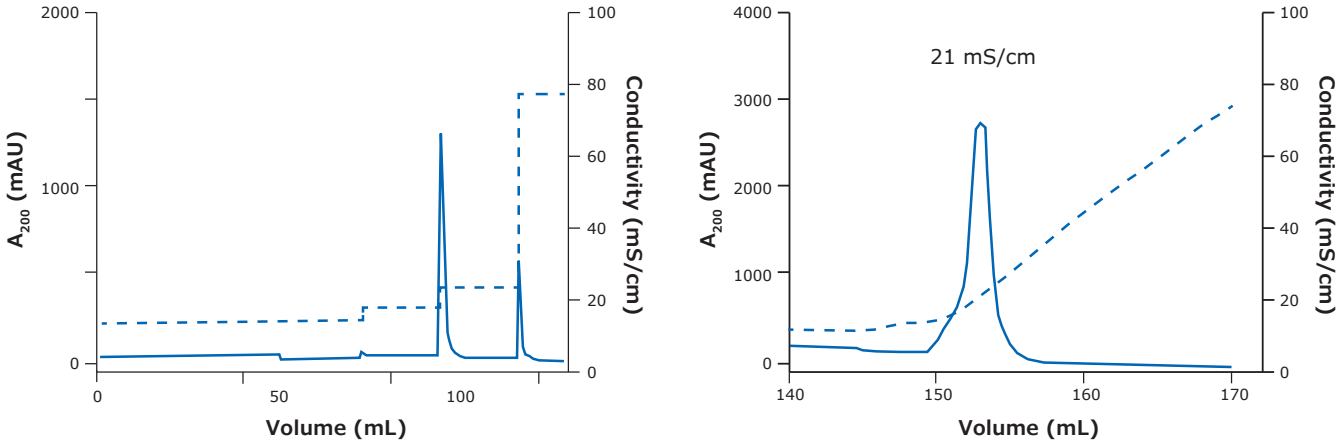


Figure 5: Chromatography of ChAdOx-2-RabG vaccine using Natrix® HD-Q membrane adsorber. (Left) Step elution of ChAdOx2-RabG with Natrix-HD Q membrane adsorber. (Right) Gradient elution ChAdOx2-RabG elution peak at 21 mS/cm.

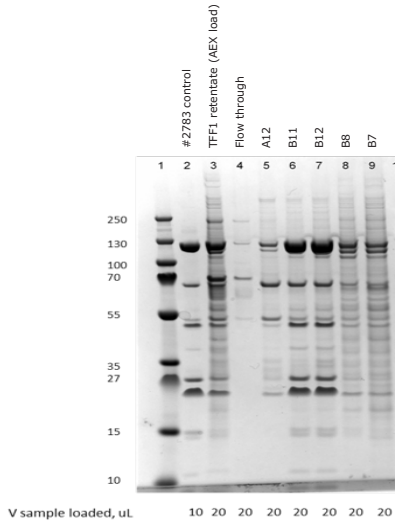


Figure 6. SDS-PAGE of ChAdOx-2-RabG vaccine after anion exchange chromatography. Elution peak samples were loaded in lines B11 and B12.

	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).

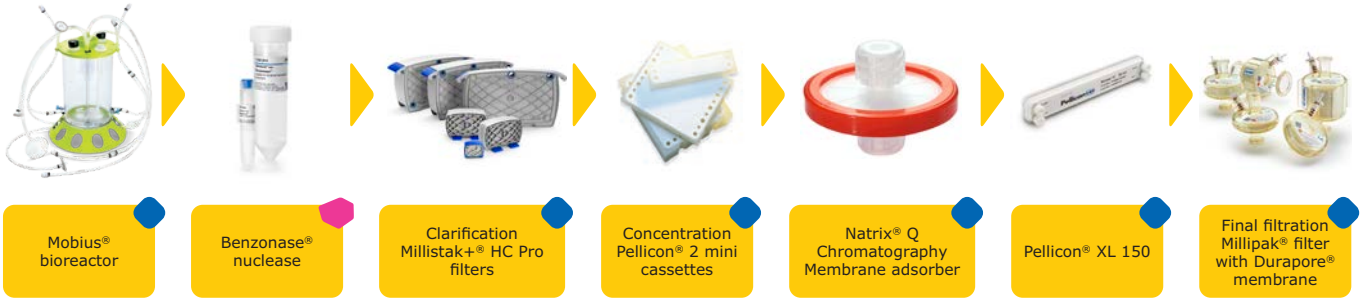


Figure 7. A optimized GMP-transferable process for adenovirus-based vaccine manufacturing.

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

Millipore® products SAFC® products

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

Neglected Tropical Diseases — Improving the Manufacturing Paradigm for a Novel Recombinant Protein Vaccine

Working in close partnership, Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine, and MilliporeSigma, the life science business of Merck KGaA, Darmstadt, Germany, are combining their bioprocess and engineering expertise to advance and optimize the manufacturing process for a promising new preventive recombinant protein-based vaccine, to end the scourge of Schistosomiasis, one of the world's most devastating and pervasive parasitic infections and neglected tropical diseases.

Peter Hotez, Texas Children's Hospital Center for Vaccine Development, Baylor College of Medicine

Maria Elena Bottazzi, Texas Children's Hospital Center for Vaccine Development, Baylor College of Medicine

Ranjeet Patil, MilliporeSigma, the life science business of Merck KGaA, Darmstadt, Germany

Bart Fryszczyn, MilliporeSigma, the life science business of Merck KGaA, Darmstadt, Germany

Introduction

Schistosomiasis is a chronic parasitic infection that ranks second only to malaria on the list of the world's most devastating infectious diseases, in terms of its public health and socioeconomic impact, according to the World Health Organization (WHO; Geneva, Switzerland). At any time, this neglected tropical disease affects an estimated 200 million people in the world's most poverty-stricken regions, with 280,000 people dying each year from Schistosomiasis-related health issues.

Among its major effects, Schistosomiasis causes chronic developmental disabilities, cognitive delays in children, severe end organ damage to the intestines, liver, and urinary tract in adolescents adults, as well as bladder cancer. In girls and women, genital Schistosomiasis may represent the most common gynecologic condition on the African continent, where it is also a leading co-factor in its HIV/AIDS epidemic.

Schistosomiasis is most prevalent across sub-Saharan Africa and the Middle East, Brazil and Venezuela in Latin America, and Philippines and China in East Asia. Schistosomiasis transmission has been reported in 78 countries. People living in areas of extreme poverty are exposed to, and infected by, the parasitic flatworms in their infective stages during routine agricultural, domestic, occupational and recreational activities involving freshwater lakes and rivers, making it impossible to break the cycle of ongoing transmission and reinfection, even for those who have been treated for the infection. (for more, see Box1, Schistosomiasis: A Primer, on page 25). It's notable that Schistosomiasis was recently noted to have emerged in Corsica, France[1], possibly as a consequence of human migrations from Africa or climate change. There are concerns that through climate change, additional sites for schistosomiasis emergence are possible[2].

Schistosomiasis: A primer

Schistosomiasis, also known as bilharzia, is caused by one of six different species of parasitic trematode worm Schistosoma, with the majority of the cases either infected with *Schistosoma mansoni (approximately one third of the global human cases), Schistosoma haematobium (two thirds), with one percent of individuals affected by Asian schistosomiasis caused by Schistosoma japonicum or Schistosoma mekongi* (Figure 1).

People become infected when they come in contact with the larval forms of these parasitic flatworms, which reproduce asexually in freshwater snails and are eventually released into the water in large numbers as infective larval schistosomes (called cercariae). These schistosomes swim freely in lakes and rivers and burrow into the skin of a human host in lakes and rivers, migrating through the blood stream and lungs to infect various organs, where it matures into another stage (schistosomula).

The cycle of transmission continues when people suffering from Schistosomiasis defecate or urinate into freshwater bodies, and the eggs are returned to the water, to be taken up by freshwater snails and later released into the water as parasitic larvae that wait for their next human host.

Long-term impact

Worm infestation can take a urogenital form damaging the kidneys, bladder, and female genital tract (from S. haematobium), or an . intestinal form (caused by *S. mansoni* and the other species of Schistosoma worm), damaging the liver and spleen over time. *S. haematobium* is also a recognized carcinogen leading to bladder cancer in Africa and the Middle East[13].

Chronic schistosomiasis is linked with numerous disease sequalae (TABLE), especially in children and adolescents. The growing burden in human hosts leads to inflammation, granulomas and host fibrosis, immune reactions and progressive organ damage, causing malaise, lethargy and failure to thrive (including both physical and cognitive deficits). Schistosomiasis is a leading cause of pediatric intellectual and cognitive deficits[14], especially in Africa, the Middle East, and Brazil.

Schistosomiasis also inflicts great social stigma (particularly on women), and the worm burden is thought to compromise the immune system, making the host more susceptible to other infections. According to WHO, Schistosomiasis is thought to be an important co-factor in the spread of HIV/AIDS in developing nations.

Major clinical sequalae of Schistosomiasis in Africa, Middle East and Latin America

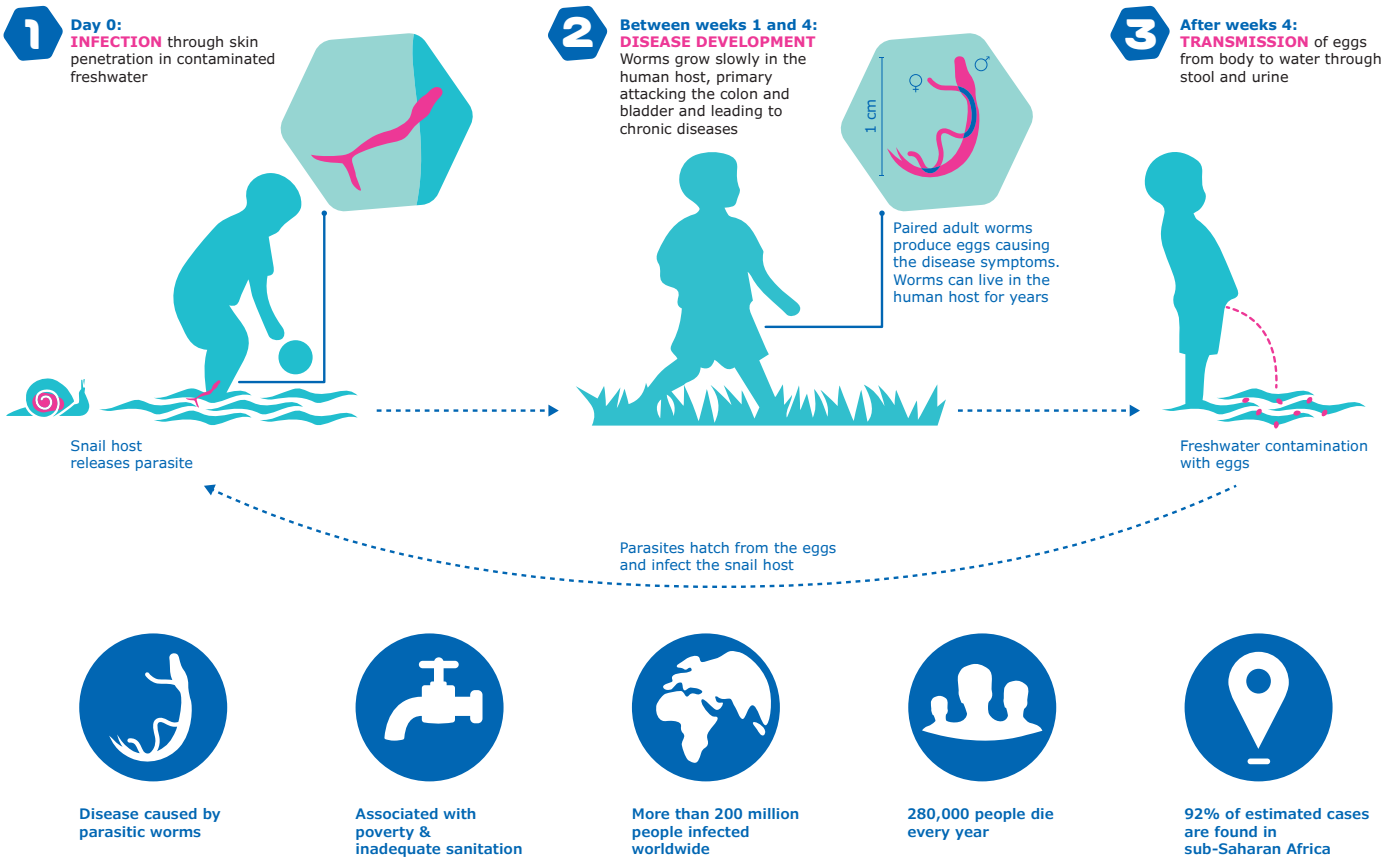
	Schistoma haematobium (2/3 of global cases)	Schistosoma mansoni
Pediatric cognitive and growth delays from anemia and inflammation	•	•
Hematuria, hydronephrosis, renal failure	•	
Bladder cancer	•	
Female genital Schistosomiasis	•	
Intestinal and liver fibrosis		•
Fulminant liver disease with hepatitis C co-infection		•

Today, the prevailing treatment option for persons with Schistosomiasis is the use of a medication called praziquantel (PZQ), which targets the parasitic worms, larvae and eggs. This acylated quinoline-pyrazine treatment therapy has a proven therapeutic and safety index. Treatment with PZQ has been the cornerstone of the WHO-promoted global strategy against Schistosomiasis for several decades.

While this therapy is highly effective toward reducing the worm burden in afflicted patients, it cannot alone eliminate Schistosomiasis for a variety of reasons[3]:

- PZQ may not be effective in all patients, and it does not kill developing larvae
- It does not prevent reinfection, as humans continue to be exposed to infected waterways
- The medicine is still not available for everyone; In fact, today only an estimated 20% of school-age children who need PZQ currently receive it
- Public health experts worry that continued reliance on PZQ raises the risk of drug-resistant parasites emerging over time

Figure 1:
Schistosomiasis Propagation and Consequences



The effort to develop a Schistosomiasis vaccine

The development of a viable, affordable vaccine against Schistosomiasis is a critical objective in ongoing efforts to break the cycle of chronic reinfection, and end organ damage, cancer and female genital Schistosomiasis, particularly in poor communities that do not have access to safe drinking water, adequate sanitation and a viable personal hygiene infrastructure. Healthcare experts anticipate that the best long-term strategy will involve routine vaccination coupled with periodic drug treatment using PZQ.

This White Paper discusses the Schistosomiasis vaccine candidate *Sm*-TSP-2, and efforts that are underway to improve and optimize its initial production process, to make it viable and sustainable at a commercial scale. The *Sm*-TSP-2 vaccine candidate was developed by a consortium of partners and team of scientists spearheaded by the product development partnership (PDP) named Texas Children's Hospital Center for Vaccine Development (TCH-CVD), in the National School of Tropical Medicine at Baylor College of Medicine, and it has shown promising results in early-phase clinical trials[4].

The *Sm*-TSP-2 vaccine antigen is a 9-kilodalton (kDa) recombinant protein that corresponds to a surface protein from the worm *Schistosoma mansoni* (*S. mansoni*), which is one of six species of trematode worm of the genus Schistosoma. *S. mansoni* is to blame for the intestinal/liver form of Schistosomiasis, and is one of the two species that is responsible for approximately one-third of all Schistosomiasis cases, including all of the cases that occur in the Americas[5].

Through the successful application of RNA interference (RNAi) and other disruptive technologies, the PDP teams were able to ascribe special functions to the *Sm*-TSP-2 molecule and improve its role in reducing parasite survival [6, 7, 8]. The molecule has been scaled up for production and further testing.

Preclinical studies and early-phase (Phase Ib) clinical trial testing to assess safety, reactogenicity, immunogenicity and ascending dose of the candidate vaccine, with or without adjuvant (AP 10-701) have shown that vaccination using this recombinant protein sub-unit can induce antibody and cellular immune response against intestinal Schistosomiasis and substantially reduce the worm burden in healthy exposed adults, in an endemic area of Brazil[9–12].

Improving the *Sm*-TSP-2 vaccine-production platform

The initial production process developed by the PDP TCH-CVD team was suitable to produce the small quantities needed for early-phase laboratory testing. However, to make the process amenable for larger-scale production, TCH-CVD has partnered with MilliporeSigma.

Through this collaborative partnership, a team of bioprocessing-oriented scientists, engineers and economists from MilliporeSigma has been working in close technical collaboration with TCH-CVD to advance the following technical and business objectives:

- Conduct a thorough technical and economic review of the overall process and a critical evaluation of each step, to identify technical and economic bottlenecks
- Investigate, implement and validate several state-of-the-art technology alternatives to improve the performance of key unit operations
- Streamline the overall system and reduce the number of unit operations, using principles of process intensification and improved engineering integration
- Develop a robust, streamlined production paradigm that maximizes production yield of the vaccine antigen at commercial scale
- Reduce capital and operating costs (including materials and labor), with a goal of minimizing the per-unit cost of the final vaccine (using the benchmark cost of $1/dose as a target)
- Develop a detailed lifecycle cost analysis for the final production paradigm, to both quantify efficiency gains and cost savings resulting from specific technology upgrades, and establish a realistic per-dose cost basis for the final vaccine product
- Carry out robust economic analysis and modeling to identify not only the direct cost savings of the system upgrades, but predict potential longer-term socioeconomic and health-related savings that would result from mass immunization using this candidate Schistosomiasis vaccine

Cost containment is especially critical when it comes to neglected tropical diseases like Schistosomiasis, as mass-vaccination campaigns often aim to administer millions of doses through programs that are typically paid for by resource-constrained local governments, non-governmental organizations (NGOs), and public/private partnerships. For more discussion on the economic impact of the technology advancements discussed here, see the Box, Understanding the Vaccine's Full Economic Impact, on page 29.

Sm-TSP-2 initial vaccine-production process

TCH-CVD initial pre-clinical production route required the following steps:

- Fermentation in a yeast-based bioreactor
- Dilution of the viscous fermentation broth (which has 30% suspended solids content), using buffering agents to dilute and wash the stream in successive steps, in order to minimize fouling the hollow-fiber-membrane filtration and resin-bead chromatography steps that follow
- Clarification, purification and volume reduction of the stream via a two-step filtration process
- Separation, concentration and capture of the vaccine antigen from the clarified stream using several chromatography steps
- Elution of the packed chromatography columns to release and harvest the antigen product that was captured by the chromatography resin beads
- Formulate the antigen into a final injectable vaccine formulation

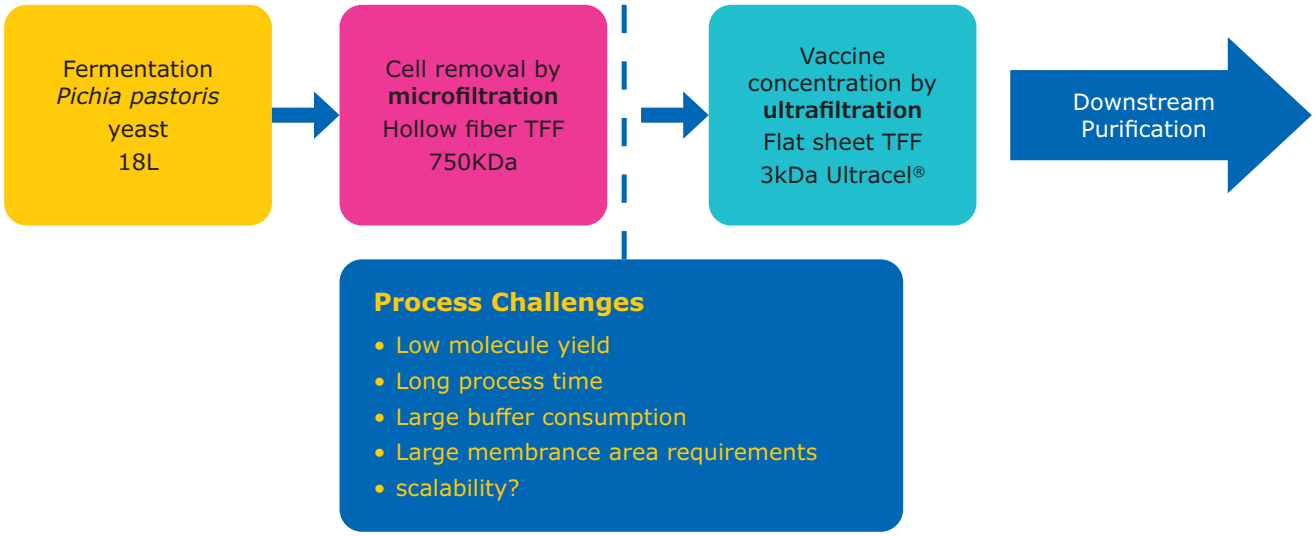
In the original process (Figure 2), the yeast-based bioreactor yields 18 liters per batch, with a suspended-solids content of 30%. To dilute and wash this stream ahead of the downstream filtration process, 12 liters of buffer must be added to each batch, to enable clarification using the two-step filtration process (to condition the stream ahead of the downstream chromatography process):

- *Step 1. Microfiltration using a hollow-fiber-membrane module.* This step removes unwanted cells by microfiltration, and is used to concentrate the expanded volume (after the 17-liter batch is expanded by adding 12 liters of added buffering agent) back down to the target volume of 12 liters
- *Step 2. Ultrafiltration using a flat-sheet, tangential flow filtration (TFF) module.* This step is used to further concentrate the batch down to the final target volume of 7.2 liters

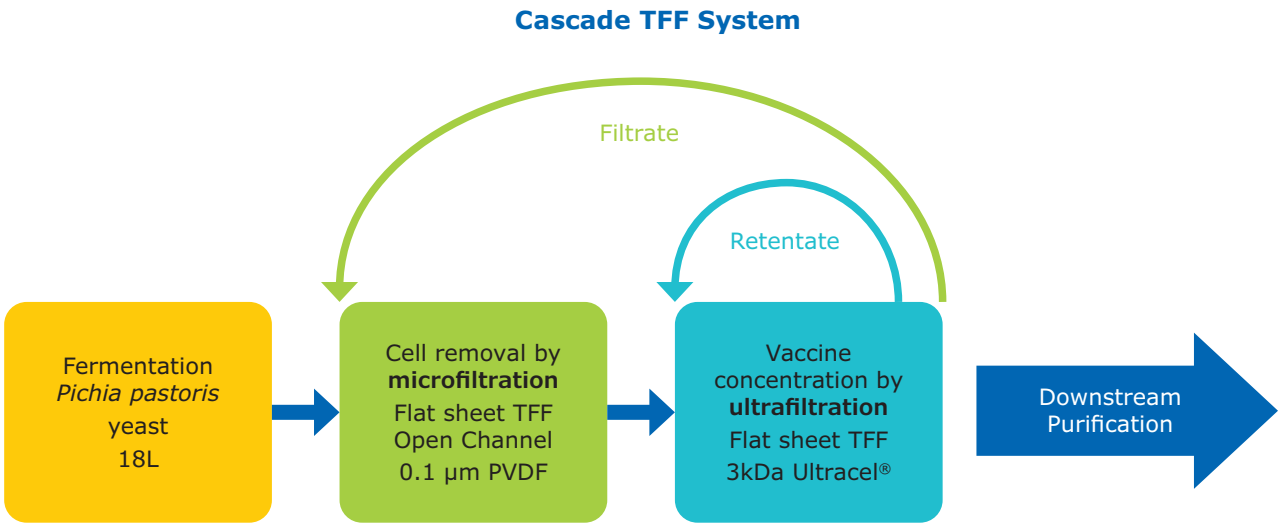
This multi-step clarification process, involving successive cycles of dilution and washing, proved to be inherently inefficient, resulting in relatively large losses of the target vaccine antigen at each step. The initial production process produced a purified stream of *Sm*-TSP-2 that had a yield of just 31% target antigen at 97% purification.

Figure 2:

Previous Clarification Process



Revised Clarification Process



Process Improvements

- Reduce complexity due to combining previously separate TFF steps
- Fouling at MF step is reduced due to continuous dilution of feed by permeate buffer recycled from the UF step
- Reduce buffer use
- Smaller membrane area requirements
- 1.7X higher concentration factor achieved
- Shorter Process Time (Shorter Suite Time)
- Smaller Manufacturing Footprint

Understanding the vaccine's full economic profile

Neglected tropical diseases often do not get the attention they need from large, multinational vaccine developers, because such products rarely provide a strong business case or sufficient profit motive to satisfy corporate objectives or shareholder expectations with regard to return-on-investment (ROI). Instead, vaccines and therapeutic interventions aimed at neglected tropical disease often rely on altruistic, humanitarian drivers, and are thus more likely to be championed by NGOs and public-private partnerships, such as the one described here. As such, the need to minimize overall production costs through engineering advances and optimization efforts is of paramount importance.

During such efforts, ongoing economic analysis and modeling should strive to quantify not only the cost savings that can result from manufacturing advances, but to the potential large-scale economic impact that can result from the successful treatment of chronic, debilitating and life-threatening conditions. Such savings come in the form of offset healthcare costs, reduced morbidity and mortality, and increased economic productivity for the community at large. Such a robust economic assessment provides a strong "artificial business case" that can help to entice private-public investment and government commitment to address neglected topical diseases.

Experts in MilliporeSigma's Value Management Center (VMC) work closely with drug- and vaccine developers, applying principles of Lean Methodology and Value Stream Mapping to evaluate processes holistically, and develop a business case that quantifies:

- The net cost reduction and financial impact on per-dose vaccine cost associated with the proposed technology improvements and process-optimization efforts
- The potential economic impact of shorter production timelines, higher production yields, lower capital and operating costs, improved speed to clinic, simplified regulatory compliance and so forth
- The potential socio-economic impact of the proposed upgrades to an established process in terms of the socioeconomic impact and offset healthcare costs that arise due to expanded access to the vaccine or lifesaving therapy
- Guidance and best practices to inform the selection of the most appropriate technologies for future applications

When MilliporeSigma's VCM team analyzed the process upgrades being pursued for *Sm*-TSP-2 Schistosomiasis vaccine, yielded demonstrable cost savings. Going forward, VCM analysis will confirm the anticipated economic impact of the chromatography-related advances that are currently being explored.

As shown in Figure 3, the process revisions discussed here yielded direct economic impact—specifically by eliminating the need to add buffering agents to dilute the bioreactor supernatant through the use of a state-of-the-art cascade TFF filtration and clarification process (A commercial-scale version of the initial process would easily have required thousands of liters of buffering agent per year). The team used the following assumptions:

- Six bioreactor runs per year
- The Pellicon® and Prostak® filter modules used in the new cascade TFF filtration process can each be used for 30 production runs before they need to be replaced

The upgraded filtration and clarification system alone (and the elimination of the dilution step needed in the original process), yielded the following cost reductions:

- *Cost of chemicals and consumables* — reduced by 98% compared to the initial process
- *Cost of labor* — reduced by 38%
- *Overall annual production cost* — reduced from $102,668 to $65,351, yielding a net savings of $37,317 per year
- *Process cost savings* — reduced by 36%
- *Amount of buffering agent required* — reduced by 88%
- *Labor hours* — down by 38%
- *Increased product recovery* — The revised process is able to recover 93% of the Sm-TSP-2 produced in the bioreactor (up from just 31% using the initial process)
- *Increased total yield* — These improvements allow the process to produce an additional 34 g of final drug product per batch run at commercial scale — representing a 36% increase in yield compared to the original process

Figure 3:
Increasing Access to Vaccine
Economic Impact of Process Revisions

Baseline Modelling

- Runs per Year = **6**
- Filter Reuses (Pellicon® cassette & Prostak® module) = **30**
- Filter Reuses (Hollow Fiber) = **1**
- Labor Hours per Run (Old) = **8**, (New) = **5**
- Buffer per Run (Old) = **92L**, (New) = **11L**
- Buffer Cost = **$0.50/L**
- Volume per Run = **20L**
- Titer = **1g/L**
- Product Recovery (Old) = **64%**, (New) = **93%**

Output

Cost Comparison ($/year) - HARD			
Unit Operation	**Old Process**	**New Process**	**% Difference**
Chemicals and Consumables	$ -35,468	$ -851	-98%
Labor	$ -7,200	$ -4,500	-38%
Grand Total	$ -102,668	$ -65,351	-36%
Estimated Hard Savings ($/year)		$ 37,317	

Business Impact

- 36% Process Cost Savings $$$
- 88% of Buffer Saved $
- 38% Labor Hours Saved $
- **Potential for an Additional 34g of Final Drug Product (36% Increase) $$$$$**

*** **VALUE IMPACT = $**

Limitations of the initial process

To make the production process technically feasible and economically viable for commercial-scale production, the TCH-CVD/MilliporeSigma team needed to implement technology and design-based upgrades to overcome several key limitations of the initial process:

- *Low molecule yield.* The initial, multi-step process was only able to achieve a protein-recovery yield of 31%
- *Long processing times, involving multiple unit operations.* This makes the process more complicated and less amenable to localized production in developing nations.
- *Large consumption of buffer fluids for dilution and washing.* The need to dilute the bioreactor supernatant creates inefficiencies, as it calls for an expanded fluid volume, longer processing times, consumption of costly buffering fluids, and reduced overall yield (as product losses occur at each of the successive dilution and volume-reduction steps)
- *Large membrane area requirements to ensure sufficient clarification of the fermentation broth with its relatively high viscosity and suspended solids content.* This adds capital costs and operating complexity to the system
- *Scalability issues.* Certain technology choices in the initial system (such as hollow-fiber-membrane filtration) do not scale up reliably

An analysis of the mass balance and purity of the *Sm*-TSP-2 produced using the initial multi-step process analysis[9] confirmed that major yield losses occur during two key steps — downstream clarification step of the fermentation broth from the bioreactor, and the multi-step chromatography process used for antigen capture. Using the amount of *Sm*-TSP-2 in the supernatant from the bioreactor as the basis (100% of the starting material), the yield fell to 79% as the percentage of total after the first filtration step, and 64% as the percentage of total after the second filtration step, and just 33% as the percentage of total after elution from the chromatography process.

Improvements focus on three areas

To advance a commercial-scale cGMP-compliant manufacturing process, the TCH-CVD/MilliporeSigma team has focused its initial efforts on improving several key processing steps and reducing the total number of unit operations that are responsible for the largest productivity penalties and yield losses:

- The clarification process that is used to clarify the fermentation broth (which has a high suspended-solids content of 30%), and reduce its volume
- The chromatography steps that are used to separate and harvest the vaccine antigen from the clarified fermentation broth
- The additives needed for the final formulation were revised, to make them more suitable for large-scale production.

Each of the three major upgrades the team is making to the initial process is discussed in greater detail here.

Upgrade 1. Using cascade tangential flow filtration (TFF) for clarification and volume reduction.

To overcome the limitations of the hollow-fiber membrane filtration system used in the original process, the team evaluated the use of a cascade tangential flow filtration (TFF) system (Figure 4). Such a system carries out two TFF steps in a single unit operation. During operation, the permeate from the second TFF step is recycled continuously to the inlet of the first TFF step, to help wash and dilute the incoming, high-solids-content fermentation broth enough to enable filtration without high consumption of buffer.

The cascade TFF system used in the upgraded process combines MilliporeSigma's open-channel, membrane-based Prostak® microfiltration modules with MilliporeSigma's 3kD Ultracel® ultrafiltration modules, and these modules are operated simultaneously. The resulting design provides an inherently compact, streamlined approach, which allows the upgraded process to efficiently concentrate each 18-liter supernatant batch to 8 liters, and eventually to 4.3 liters. This allows a cleaner, more-concentrated stream to be delivered to the downstream chromatography step — without the need to first expand the stream by first adding (and later removing) 12 liters of buffer fluid.

The cascade TFF system not only improves the clarification efficiency and reduces the total volume of buffer fluid needed, but it reduces capital and operating costs and processing time, reducing the clarification and filtration time from two shifts to a single shift. This upgrade also enables a smaller downstream chromatography system to be used (thanks to enhanced volume reduction during the filtration step).

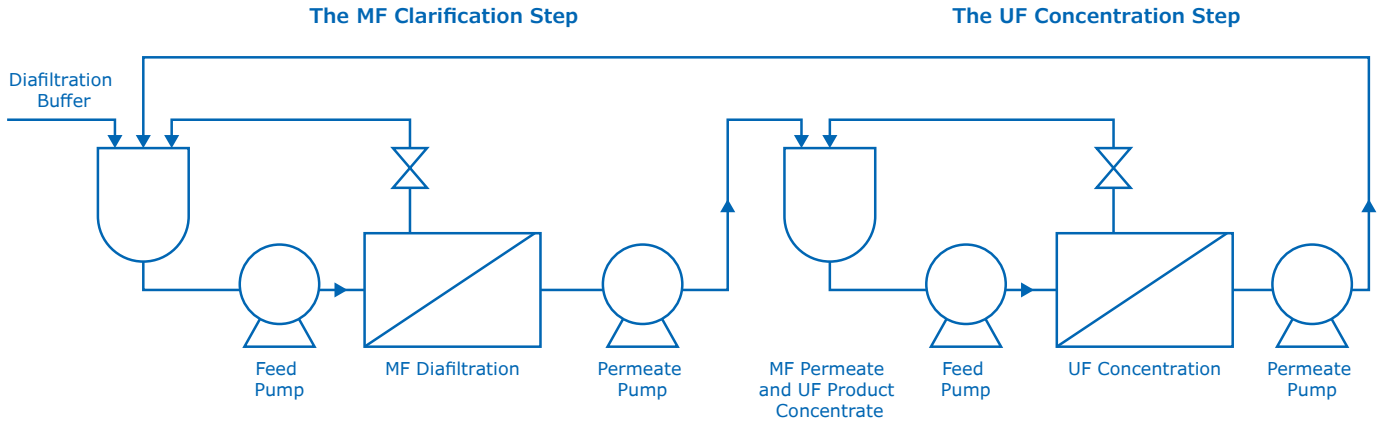
Upgrade 2. Improving the chromatography step

In the original process, the *Sm*-TSP-2 antigen binds to a cation exchange resin (based on an older, agarose-based chromatography resin), during a traditional bind-and-elute chromatography step. Majority of host cell proteins (Host cell proteins and other impurities) are collected in the column flow-through. After washing the column with Succinic acid buffer at pH 6 (~15 mS/cm), the Sm-TSP-2 molecules bound by the chromatography resin is eluted with a linear gradient using 500 mM sodium chloride.

While suitable to produce small-scale quantities of the antigen needed for the early, small-scale testing, the chromatography step in the original process provides relatively poor recovery yield (on the order of 50%). And, to cope with insufficient impurity removal, a second chromatography step is required for polishing.

Figure 4:
Sm-TSP-2 Production Cascade TFF system

A Typical Cascade TFF System



Rather than two separate TFF operations, combine micro- and ultrafiltration into **single, linked process**

The MilliporeSigma team is working to apply its extensive product, engineering, design and troubleshooting expertise to evaluate several competing options for improving chromatography step used in the initial system design. The initial process uses a two-step, resin-bead-based chromatography system. Ongoing investigation is under way to explore and test a variety of options, with a goal of both streamlining and maximizing product capture and reducing the two-step chromatography option to a single step.

Importantly, the redesign of the clarification process is already delivering a smaller, cleaner stream to the downstream chromatography process. Using today's state-of-the-art resins and column designs, the goal is to reduce the number of columns, and to maximize the number of bind-and-elute cycles before the resin must be replaced. The team expects that the final design upgrades in these two areas will help to improve the product-capture yield of the chromatography step (which is roughly 50% in the initial process), and further reduce the capital and operating costs of the commercial-scale manufacturing process *Sm*-TSP-2, enabling a more sustainable and appropriate design for developing nations aiming for localized Schistosomiasis vaccine production.

Upgrade 3. Focus on the vaccine formulation

The initial vaccine formulation produced by the TCH-CVD team used 10-millimolar (mM) of the buffering agent imidazole, 15% sucrose and 2 mM phosphate at pH 7.4. While this formulation was suitable for preclinical and early-phase testing, imidazole is not suitable for use in parenteral formulations, and is not readily available in GMP grade — potentially hindering the regulatory approval of the final vaccine formulation.

After investigating suitable alternatives, the MilliporeSigma experts recommended replacing imidazole with histidine as the buffering agent of choice, to stabilize the proteins through mild buffering capacity and minimize unwanted protein-protein interactions that can lead to aggregation. The improved formulation is now comprised of 20 mM of histidine, 15% sucrose and 4 mM of phosphate at pH 7.4.

Histidine is a similar molecule to imidazole, with compatible biochemical aspects to imidazole (in terms of stability, pH, and other product characteristics), and functions similarly in terms of stabilizing the antigen. However, unlike imidazole, histidine has already been used in other vaccines that have transitioned successfully to the clinic (having received FDA approval with regard to safety and efficacy), and it is readily available in both parenteral formulations and GMP grade. These attributes should help *Sm*-TSP-2 Schistosomiasis vaccine to avoid potential regulatory hurdles.

Closing thoughts

While many factors will contribute to technical and economic viability of any commercial-scale vaccine-production process, the ability to optimize key parameters as early as possible in the process-development phase can have the largest long-term impact in terms of locking in operating flexibility, reducing overall costs, improving the likelihood of regulatory approval and minimizing the per-dose vaccine cost. The time to explore and validate relevant process improvements is when the prototype production process is still at small scale, as it always more difficult and more costly to revise the process later. Such performance objectives are essential for any biopharmaceutical process but become even more critical to enable the localized production and widespread administration of vaccines and lifesaving therapies aimed at reducing neglected tropical diseases in developing nations.

As demonstrated here, the ability to use state-of-the-art technology substitutions and advanced design concepts has helped the TCH-CVD team to improve clarification and volume reduction, reduce fouling in filtration and chromatography steps, reduce the volume of buffer fluids and the overall membrane required, reduce and simplify the final system design, and improve overall antigen-recovery rates, concentration factor and purity thresholds, paving the way for a commercial-scale, cGMP-compliant production paradigm for this candidate Schistosomiasis vaccine.

The long-term technical collaboration between the TCH-CVD and MilliporeSigma experts continues to provide demonstrable advantages for both partners, allowing all stakeholders to bridge scientific and engineering knowledge gaps (and essentially establishing a *de facto* scientific advisory board) that exploit synergies to overcome critical hurdles that can hamper process development and scaleup, product formulation, critical economic assessment and ongoing training, leveraging that collaborative effort to bring promising, affordable vaccines and lifesaving therapies to market more quickly.

References

1. Le Govic Y, Kincaid-Smith J, Allienne J, Rey O, de Gentile L, Boissier J. Schistosoma haematobium–Schistosoma mansoni Hybrid Parasite in Migrant Boy, France, 2017. *Emerg Infect Dis.* 2019;25(2):365-367. https://dx.doi.org/10.3201/eid2502.172028; https://www.ncbi.nlm.nih.gov/pubmed/30526763
2. Blum AJ, **Hotez** PJ. Global "worming": Climate change and its projected general impact on human helminth infections. *PLoS Negl Trop Dis.* 2018 Jul 19;12(7):e0006370; https://doi.org/10.1371/journal.pntd.0006370
3. Lo NC, Addiss DG, **Hotez** PJ, King CH, Stothard JR, Evans DS, Colley DG, Lin W, Coulibaly JT, Bustinduy AL, Raso G, Bendavid E, Bogoch II, Fenwick A, Savioli L, Molyneux D, Utzinger J, Andrews JR. A call to strengthen the global strategy against schistosomiasis and soil-transmitted helminthiasis: the time is now. *Lancet Infect Dis.* 2017 Feb;17(2):e64-e69. doi: 10.1016/S1473-3099(16)30535-7. Epub 2016 Nov 30. Review. https://www.ncbi.nlm.nih.gov/pubmed/27914852
4. Hotez PJ, Bottazzi ME, Bethony J, Diemert DD. Advancing the development of a human Scistosomiasis vaccine. *Trends Parasitol.* 2019 Feb;35(2):104-108. doi: 10.1016/j.pt.2018.10.005. Epub 2018 Nov 16. https://www.sciencedirect.com/science/article/pii/S1471492218302368
5. Baylor College of Medicine, Texas Children's Hospital Center for Vaccine Development, Schistosomiasis Vaccine; https://www.bcm.edu/departments/pediatrics/sections-divisions-centers/tropical-medicine/research/vaccine-development/schistosomiasis
6. Merrifield, M., Hotez, PJ and others, Advancing a Vaccine to Prevent Human Schistosomiasis, *Vaccine*, Volume 34, Issue 26, 3 June 2016, 2988-291; http://doi.org/10.1016/j.vaccine.2016.03.079. https://www.ncbi.nlm.nih.gov/pubmed/27036511
7. Tran MH, Freitas TC, Cooper L, Gaze S, Gatton ML, Jones MK, Lovas E, Pearce EJ, Loukas A. Suppression of mRNAs encoding tegument tetraspanins from Schistosoma mansoni results in impaired tegument turnover. *PLoS Pathog.* 2010 Apr 15;6(4):e1000840. doi: 10.1371/journal.ppat.1000840; https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1000840
8. Jia X, Schulte L, Loukas A, Pickering D, Pearson M, Mobli M, Jones A, Rosengren KJ, Daly NL, Gobert GN, Jones MK, Craik DJ, Mulvenna J. Solution structure, membrane interactions and protein binding partners of the tetraspanin sm-TSP-2, a vaccine antigen from the human blood fluke Schistosoma mansoni. *J Biol Chem.* 2014 Mar 7;289(10):7151-63. doi: 10.1074/jbc.M113.531558. Epub 2014 Jan 15.
9. Curti E, Kwityn C, Zhan B, Gillespie P, Brelsford J, Deumic V, Plieskatt J, Rezende WC, Tsao E, Kalampanayil B, Hotez PJ, Bottazzi ME. Expression at a 20L scale and purification of the extracellular domain of the Schistosoma mansoni TSP-2 recombinant protein: a vaccine candidate for human intestinal schistosomiasis. *Hum Vaccin Immunother.* 2013 Nov;9(11):2342-50. Epub 2013 Jul 30.
10. Cheng W, Curti E, Rezende WC, Kwityn C, Zhan B, Gillespie P, Plieskatt J, Joshi SB, Volkin DB, Hotez PJ, Middaugh CR, Bottazzi ME. Biophysical and formulation studies of the Schistosoma mansoni TSP-2 extraceullular domain recombinant protein, a lead vaccine candidate antigen for intestinal schistosomiasis. *Hum Vaccin Immunother.* 2013 Nov;9(11):2351-61. Epub 2013 Jul 23.
11. Tran MH, Pearson MS, Bethony JM, Smyth DJ, Jones MK, Duke M, Don TA, McManus DP, Correa-Oliveira R, Loukas A. Tetraspanins on the surface of Schistosoma mansoni are protective antigens against schistosomiasis. *Nat Med.* 2006 Jul;12(7):835-40. Epub 2006 Jun 18. https://www.ncbi.nlm.nih.gov/pubmed/16783371
12. Pearson MS, Pickering DA, McSorley HJ, Bethony JM, Tribolet L, Dougall AM, Hotez PJ, Loukas A. Enhanced protective efficacy of a chimeric form of the Schistosomiasis vaccine antigen Sm-TSP-2. *PLoS Negl Trop Dis.* 2012;6(3):e1564. doi: 10.1371/journal.pntd.0001564. Epub 2012 Mar 13. https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0001564
13. Ishida K, Hsieh MH., Understanding Urogenital Schistosomiasis-Related Bladder Cancer: An Update *Front Med (Lausanne).* 2018 Aug 10;5:223. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6104441/
14. Majid MF, Kang SJ, Hotez PJ., Resolving "worm wars" An extended comparison review of findings from key economics and epidemiological studies. *PLoS Negl Trop Dis.* 2019 Mar 7;13(3):e0006940. https://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0006940

Authors

Peter Hotez MD, PhD, FASTMH, FAAP, is Dean, National School of Tropical Medicine, Professor, Pediatrics and Molecular & Virology and Microbiology, and Co-Head, Section of Pediatric Tropical Medicine, at Baylor College of Medicine. He is also the Texas Children's Hospital Endowed Chair of Tropical Pediatrics, Co- Director, Texas Children's Hospital Center for Vaccine Development and University Professor in both the Department of Biology, Baylor University and Baker Institute Fellow in Disease and Poverty, Rice University (Houston, Tex.). Hotez serves as the Co-Editor-in-Chief, *PLoS Neglected Tropical Diseases*. He obtained his MD and PhD from Weil-Cornell Medical College and Rockefeller University, and his pediatric residency training from Massachusetts General Hospital (Harvard Medical School). He is an elected member of the National Academy of Medicine and American Academy of Arts & Sciences (Email: hotez@bcm.edu).

Maria Elena Bottazzi, Ph.D., FASTMH is Associate Dean at the National School of Tropical Medicine, and Professor, Pediatrics, Section of Pediatric Tropical Medicine & Molecular Virology & Microbiology, at both Baylor College of Medicine and Texas Children's Hospital (Houston, Tex.). She is also a Distinguished Professor in the Department of Biology, Baylor University, and serves as Co-Director, Texas Children's Hospital Center for Vaccine Development - Product Development Partnership. She also serves as Editor-in-Chief, Current Tropical Medicine Reports, Springer, U.S. She obtained her PhD from the University of Florida and her postdoctoral fellowship from University of Miami and the University of Pennsylvania.
Email: bottazzi@bcm.edu.

Ranjeet Patil, MS, is Segment Head, Vaccines and Viral Therapies, Bioprocessing, Life Sciences and Process Solutions for MilliporeSigma (Bedford, Mass.), the life science business unit of Merck KGaA, Darmstadt, Germany. He holds a BS in Biotechnology Engineering from Shivaji University (Kolhapur, Maharashtra, India) and an MS in Biotechnology from Northeastern University (Boston, Mass.).
(Email: ranjeet.patil@milliporesigma.com).

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Filtration Strategies for Optimal Development and Purification of a Foot and Mouth Disease Virus Produced in BHK21 Cells

Youness Cherradi: Senior Process Development Scientist – EMEA, MilliporeSigma
Youssef Gaabouri: Associate Director Sales, North & West Africa, MilliporeSigma
Anissa Boumlic: Head of Global Vaccine & Plasma Segments, MilliporeSigma
Nargisse El Hajjami: Associate Director Cell and Gene Therapy Segment EMEA, MilliporeSigma
Dr Leonid Dudnikov: Production Site Director, Middle East for Vaccines

Foot-and-mouth disease (FMD) is a highly contagious and sometimes fatal viral disease that affects cloven-hoofed animals. Massive vaccination with at least 80% coverage is one of the control strategies implemented to prevent virus introduction and development. FMD vaccine manufacturing follows a simple multi-step process, a general outline of which is provided in **Figure 1.**

We collaborated with MEVAC to optimize upstream and downstream processes for FMD vaccine manufacturing to establish a scalable, cost-efficient and GMP compliant process. This white paper focuses on the integration of new filtration strategies in both upstream and downstream processes.

Objective

Optimize the filtration strategies used during downstream processing of foot and mouth disease (FMD) virus vaccine to ensure cost effective and robust manufacturing

Collaborator

MEVAC (Middle East for Vaccines) is a private company in Egypt for development and manufacturing of vaccines

Results

- Cellvento® BHK-200 serum-free medium for FMDV production in BHK21 cells can easily be filtered using Millipore Express® filters for bacteria or and/or mycoplasma clearance
- High capacity and low turbidity achieved on the clarification step allowing a very cost-efficient and low footprint scale-up with the Millistak+® HC C0HC filter
- Pellicon® 2 300 kDa or 1000 kDa tangential flow filtration can be implemented for the concentration and diafiltration of the FMD vaccine
- The adjuvant filtration process which had required three different filtration steps and a total of eleven 30” filters was replaced by a single Millipore Express® PHF 0.2 µm filter

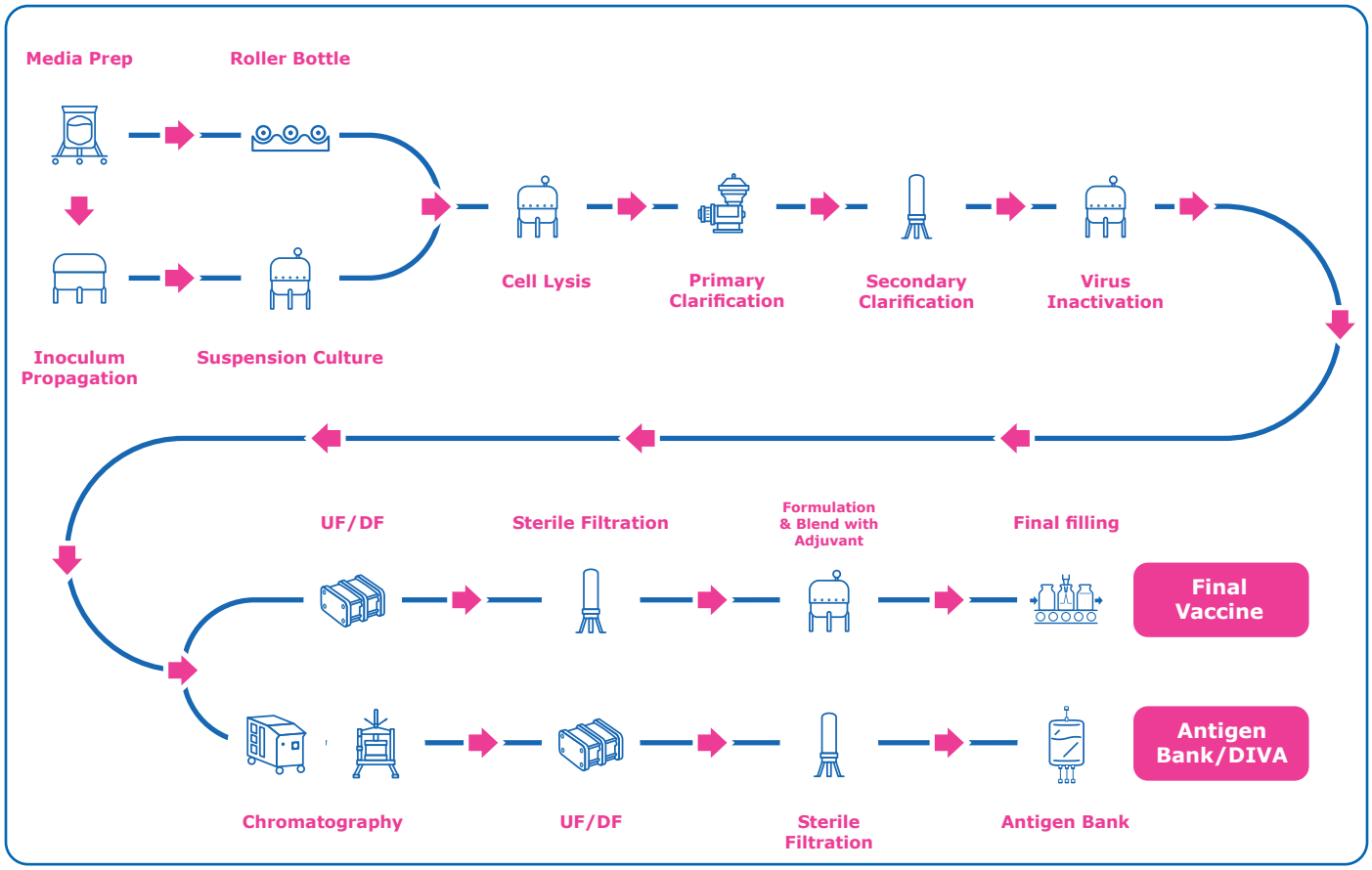

Figure 1. Typical process for manufacturing FMD vaccines.

Optimizing Media Filtration

BHK21 cells grown in suspension are typically used for production of FMD vaccines. This cell line is banked to grow in Glasgow Minimum Essential Medium (GMEM) supplemented with 5% tryptose phosphate broth (TPB) and 10% serum in spinner flasks, roller bottles, or small bioreactors.

To eliminate the risk presented by bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) agents and mycoplasma contaminations, Cellvento® BHK-200 serum free medium was used in this process. The medium is formulated without animal derived components and optimized for the culture of suspension BHK21 cells at high-density and viability with efficient propagation of viruses. Cells grown in Cellvento® BHK200 medium are first adapted via serial passaging in the medium supplemented with decreasing concentration of serum. Adapted BHK21 cells can then be grown as suspension cultures in T-flasks, shaker flasks, spinner bottles or stirred tank bioreactors with a higher growth in comparison to GMEM/TPB/serum cultures.

During preparation of the media, sterilizing-grade filtration was performed, and different filters were screened in order to define the most efficient option (**Table 1**).

Table 1. Several sterilizing-grade filters were evaluated for media preparation.

Filter	Filter details	Cat. No	Membrane area (cm²)
Durapore® 0.22 µm	PVDF (bacterial retention)	**SVGLA25NB6**	3.5
Millipore Express® SHF 0.2 µm	PES (bacterial retention)	**SGEPA25NB6**	3.5
Millipore Express® SHC 0.5/0.2 µm	PES (bacterial retention)	**SHGEA25NB6**	3.5
Millipore Express® SHR 0.1 µm	PES (bacterial, mycoplasma retention)	**SVEPA25NB6**	3.5
Millipore Express® SHR-P 0.5/0.1 µm	PES (bacterial, mycoplasma retention)	**SHVEA25NB6**	3.5

BHK200 medium was challenged against different sterilizing-grade filters for bacterial and/or mycoplasma retention **(Table 2)**. The best capacity was observed with the Millipore Express® SHF 0.2 µm and SHC 0.5/0.2 µm filters for the sterilizing-grade options; the SHC 0.5/0.2 µm filters showed no signs of plugging in comparison to the single layer Millipore Express® filter. Millipore Express® SHC and SHR-P filters have an on-board polyethersulfone membrane prefilter which protects the 0.2 or 0.1 µm membrane, respectively, from premature plugging. Similar performances were observed for the two mycoplasma-retentive Millipore Express filters. The highest Vmax™ was obtained using the SHC 0.5/0.2 µm filter.

Figure 2 shows the throughput profile over time for the five filters evaluated. A comparison of the Millipore Express® SHF 0.2 µm and SHC 0.5/0.2 µm filters showed that the single membrane provided a high flux while addition of the prefilter provides high capacity.

Table 3 summarizes filter sizing recommendations for processing 1000 L of medium and indicates that the Millipore Express® SHC 0.5/0.2 µm filter would enable a smaller footprint.

Table 2. Filtration trial results.

Prefilter	Diff. Pressure	Trial Loading	Trial Flux Decay	Vmax
	psi	L/m²	%	L/m²
Durapore® 0.22 µm	10	540.0	69.6	1782.09
Millipore Express® SHF 0.2 µm		1437.1	71.6	2080.43
Millipore Express® SHC 0.5/0.2 µm		1260.0	2.5	1758003.38
Millipore Express® SHR 0.1 µm		888.6	36.8	N/A
Millipore Express® SHR-P 0.5/0.1 µm		965.7	35.8	2685.21

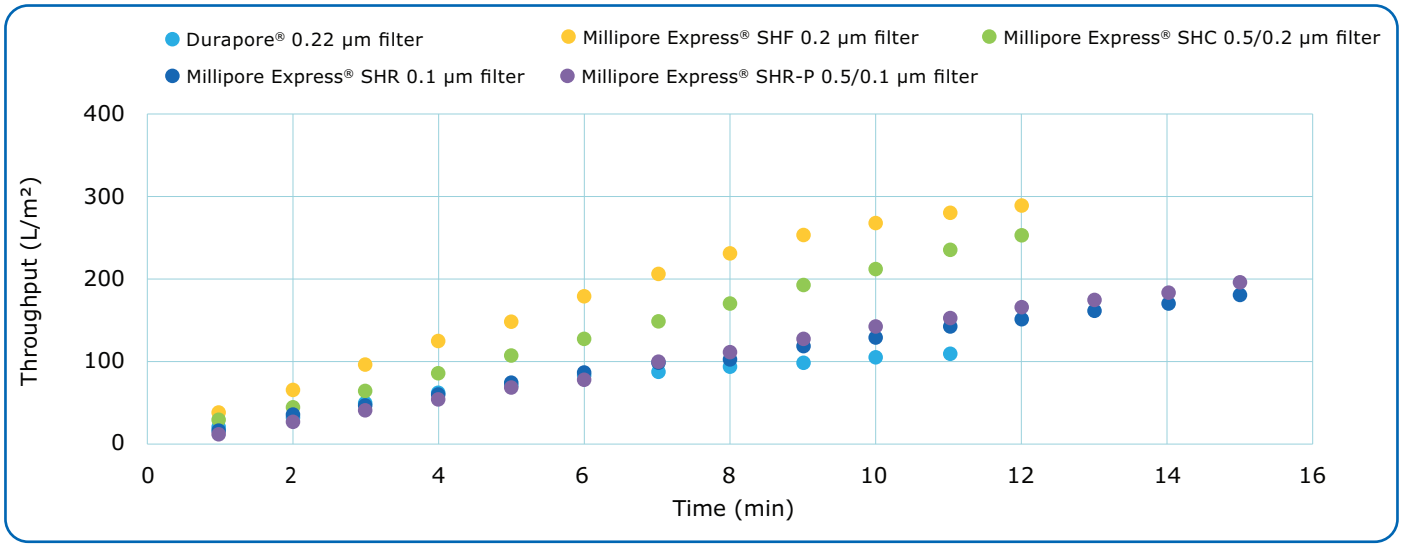

Figure 2. Throughput profile over time.

Table 3. Sizing recommendations for processing 1000 L of medium.

Filter	Batch Volume	Process Loading	A_{min} [1]	Recommended Configuration	Resultant Area	Final Safety Factor
	L	L/m²	m²		m²	x
Durapore® 0.22 µm	1000	724.6	0.71	1x OptiCap® XL20	1.38	1.9
Millipore Express® SHF or PHF 0.2 µm		925.9	0.48	1x OptiCap® XL20	1.08	2.2
Millipore Express® SHC 0.5/0.2 µm		7692.3	0.08	1x OptiCap® XL3	0.13	1.6
Millipore Express® SHR 0.1 µm		1666.7	0.32	1x OptiCap® XL10	0.60	1.9
Millipore Express® SHR-P 0.5/0.1 µm		1020.4	0.38	1x OptiCap® XL20	0.98	2.6

[1] A_{min} is the minimum calculated filter area to achieve filtration (with no safety factor)

Optimizing Clarification

The objective of clarification is to remove cell debris and contaminants and recover virus. Zonal centrifugation is commonly used for final clarification while others use a body feed sparkler assembly. Since solid content in viral vaccine harvest is low, depth filters or disc stack centrifuge typically work well for primary clarification.

Two harvests were tested for clarification with a Millistak+® HC C0HC depth filter using the pressure max and turbidity max (Pmax/Tmax) methodology at a constant flow rate; two prefilters were also evaluated **(Table 4)**. The results shown in **Figures 3** and **4** indicate that the Millistak+® HC C0HC filter was capable of processing the BHK21 cell culture in a single step, without requiring a secondary depth filter. The pressure rise occurred at 180 L/m² and turbidity remained below 15 NTU. **Table 5** summarizes the Millistak+® HC C0HC filtration results.

Table 4. Specification of the depth filter and prefilters evaluated in the study.

Filter	Filter details	Membrane area (cm²)	Cat. No
Millistak+® HC C0HC	30DE + 60DE	23	**MC0HC23CL3**
Durapore® 0.45 µm	PVDF, Bioburden Reduction	3.5	**SPHLA25NB6**
Milligard® PES 1.2/0.45 µm	PES, Bioburden Reduction	3.5	**SMP4A25NB6**

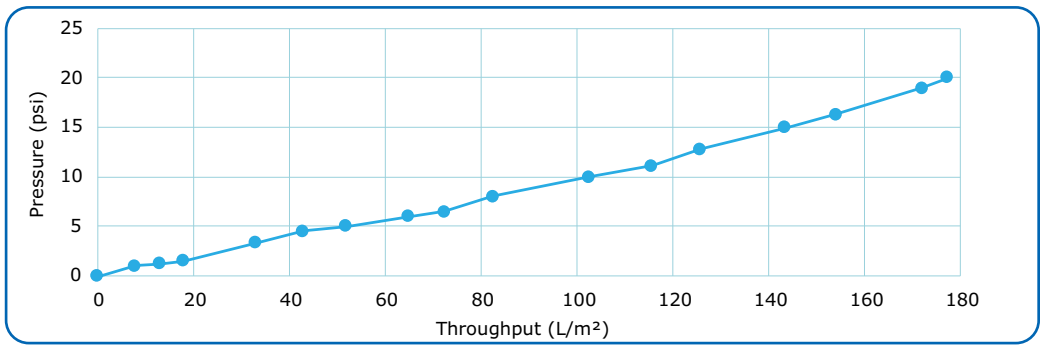

Figure 3. Millistak+® HC C0HC pressure profile.

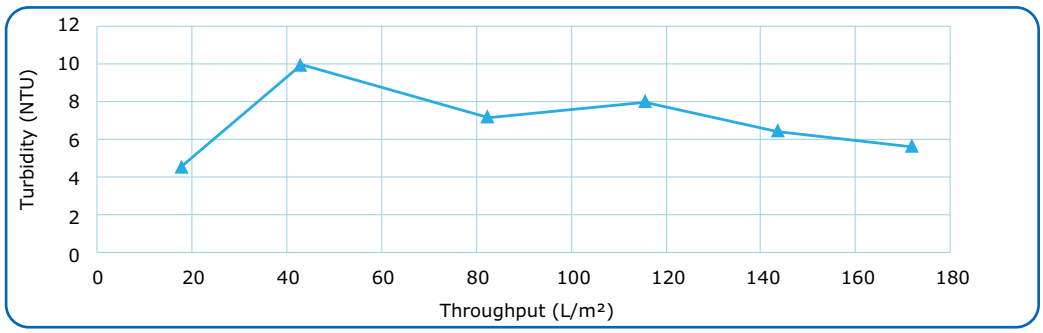

Figure 4. Millistak+® HC C0HC turbidity profile.

Table 5. Summary of Millistak+® HC C0HC filtration results.

Trial flux (LMH)	Trial loading (L/m²)	Trial endpoint inlet pressure (psi)	Harvest turbidity (NTU)	Filtrate pool turbidity (NTU)
152	177.4	20 (Pmax)	260	9.5

Filterability of the clarified harvest was also assessed on bioburden reduction membranes. Constant pressure tests were performed at 10 psi with Durapore® 0.45 µm and Milligard® PES 1.2/0.45 µm small scale filters to determine the theoretical maximum volume (Vmax™) of each solution filterable on the membrane. The initial filtrate flux (Qi) was also determined to estimate the minimum area required to filter the batch.

As shown in **Table 6**, the Milligard® PES 1.2/0.45 µm filter showed better performance than the Durapore® 0.45 µm filter and would be the preferred option for the bioburden reduction step, prior to concentration and diafiltration of the product. **Table 7** summarizes filter sizing recommendations for processing 1000 L of medium.

Table 6. Vmax bioburden reduction experiments.

Membrane filter	Membrane filter loading (L/m²)	Trial Flux decay (%)	Sterilizing-grade filter Vmax (L/m²)	Qi (LMH)
Durapore® 0.45 µm	478.9	98	545.2	23716.7
Milligard® PES 1.2 /0.45 µm	596.0	54	1667.2	25671.9

Table 7. Sizing recommendations for processing 1000 L of harvest

Filter	Amin (m²)	Suggested config.	Area (m²)	Safety Factor	Processloading (L/m²)
Millistak+® HC C0HC	5.3	7x 1.1 m²	7.7	1.5	130.0
Durapore® 0.45 µm filter	1.84	3x Opticap® XL20	3.7	2.0	268.8
Or Milligard® PES 1.2/0.45 µm filter	0.61	1x Opticap® XL20	1.2	2.0	833.3

Optimizing Concentration/Diafiltration

Tangential flow filtration (TFF) is commonly used to remove inactivating agent by diafiltration and concentration of virus.

The concentration step of the FMD vaccine production process was optimized using Biomax® membranes in a Pellicon® 2 cassette for TFF **(Table 8)**. These membranes are made of polyethersulfone which is designed to reduce non-specific protein binding and are resistant to harsh chemicals used in cleaning, biological decontamination and sanitization. Membranes are available in four screen formats: V screen (suspended), C screen (coarse), A screen (fine) and D screen (for high viscosity).

Table 8. Specifications of ultrafiltration membranes tested

Device	Membrane	Screen	Cat. No.	Area (m²)
Pellicon® 2 cassette	Biomax® 300 kDa	C	**P2B300C01**	0.1
	Biomax® 1000 kDa		**P2B01MC01**	

No pressure instability was observed with increasing permeate fluxes. Using the Biomax® 300 kDa membrane, the trial was stopped at 67.2 liter/m²/h LMH with a final transmembrane pressure (TMP) of 5.9 psi with the permeate valve completely open **(Figure 5)**. The starting TMP was 3.96 psi meaning that the trial was stopped with a 1.5x increase in pressure. For the 1000 kDa membrane, a 1.37x increase in pressure was noted at the end of the optimization study, with a permeate valve completely open. It is typical to consider pressure instability when the increase in pressure is > 1.5-2.0 and recommended to use the membranes at 75% of the maximal working flux.

Both membranes can be used with approximately the same hydraulic performances. The 300 kDa membrane tested was new while the 1000 kDa had been previously used by MEVAC for concentration without controlled monitoring of the system pressure and flowrates (feed and permeate). Performance of the 1000 kDa membrane during this optimization study can therefore be affected by previous use and membrane polarization was unbalanced.

Recommended operating parameters for the TFF step are provided in **Table 9**.

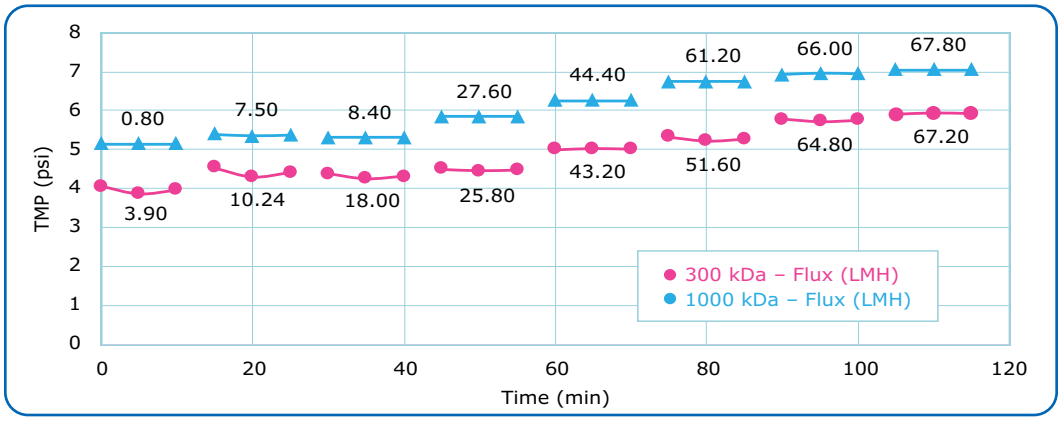

Figure 5. Results of the flux excursion study.

Table 9. Recommended operating parameters.

Membrane	Pump Flowrate	TMP (psi)	Set permeate Flux (LMH)
300 kDa	4 LMM	5	50
1000 kDa		6.5	51

Optimizing Adjuvant Filtration

Adjuvants are added to vaccine formulations to enhance the immune response and increase the level and duration of protection that is induced. Among the most used adjuvants are aluminum-based or water-in-oil or water-in-oil-in-water emulsions and lipids. Because they will be present in the final formulation, adjuvants must be sterile filtered. While it is impossible to filter an aluminum solution through a 0.22 µm sterilizing-grade filter, sterility is typically achieved through heat sterilization; sterile filtration is challenging for oil adjuvants and lipid-based formulations.

Filtration performance is affected by processing conditions, filter selection and feed stream properties. As such, careful optimization of temperature, pressure, membrane and particle size and loading is essential to establish an efficient process and assure sterility.

The initial process used for sterilizing-grade filtration of FMD vaccine adjuvants included three different steps using a total of eleven 30" filters; processing time exceeded 8 hours for a 2500L batch **(Figure 6)**.

Three filters were evaluated for the ability to filter the FMD vaccine adjuvant; the best capacity was observed with the Millipore Express® PHF 0.2 µm **(Figure 6)**. Implementation of this solution allowed compression of the adjuvant filtration train to a single 30" filter **(Figure 7)**.

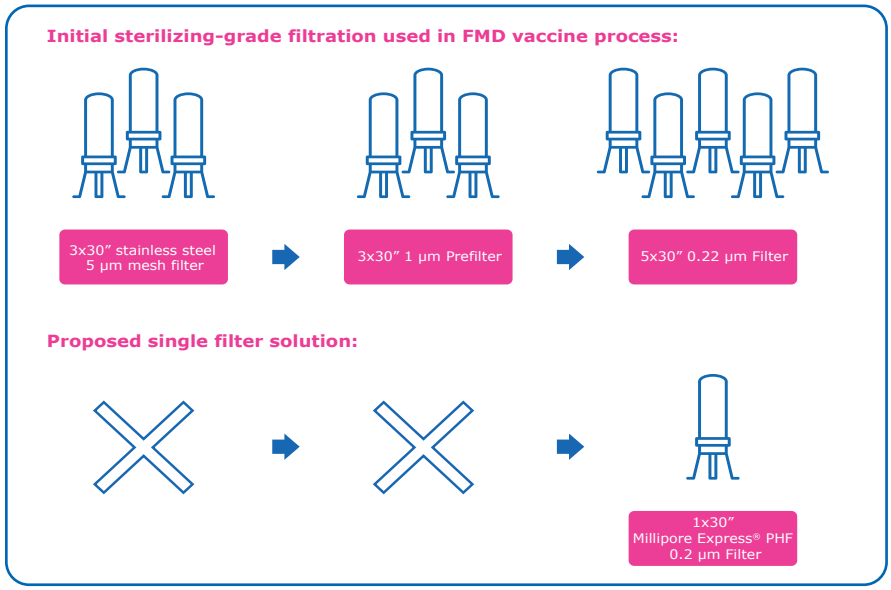

Figure 6. Initial sterilizing-grade filtration of the adjuvant used in the FMD vaccine process (A) and the new process requiring one step (B).

Table 10. FMD vaccine adjuvant filtration test results: filter details

Filter	Filter details
Durapore® 0.22 µm hydrophobic	PVDF (bacterial retention)
Millipore Express® PHF 0.2 µm	PES (bacterial retention)
Millipore Express® SHC 0.5/0.2 µm	PES (bacterial retention)

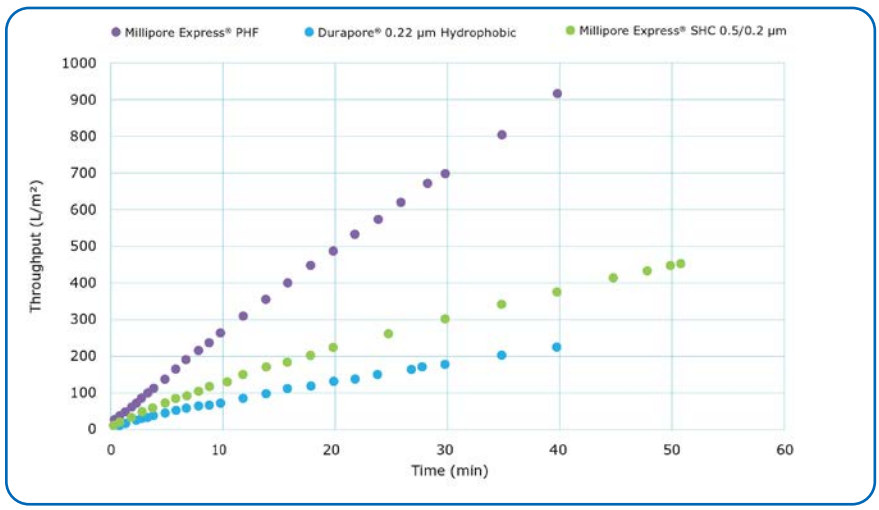

Figure 7. FMD vaccine adjuvant filtration test results

Conclusion

Incorporation of new filtration strategies delivered significant improvement to both upstream and downstream processes including media preparation, clarification, TFF and adjuvant filtration for production and purification of an FMD vaccine.

The Cellvento® BHK-200 serum free medium was easily filtered using Millipore Express® filters. The high capacity and low turbidity achieved for the clarification step allowed for a cost-efficient and low footprint scale-up using a Millistak+® HC C0HC filter while either the Pellicon® 2 300 kDa or 1000 kDa cassettes were implemented for concentration and diafiltration of the FMD vaccine. Use of a Millipore Express® PHF 0.2 µm filter allowed compression of the adjuvant filtration train from eleven to one 30".

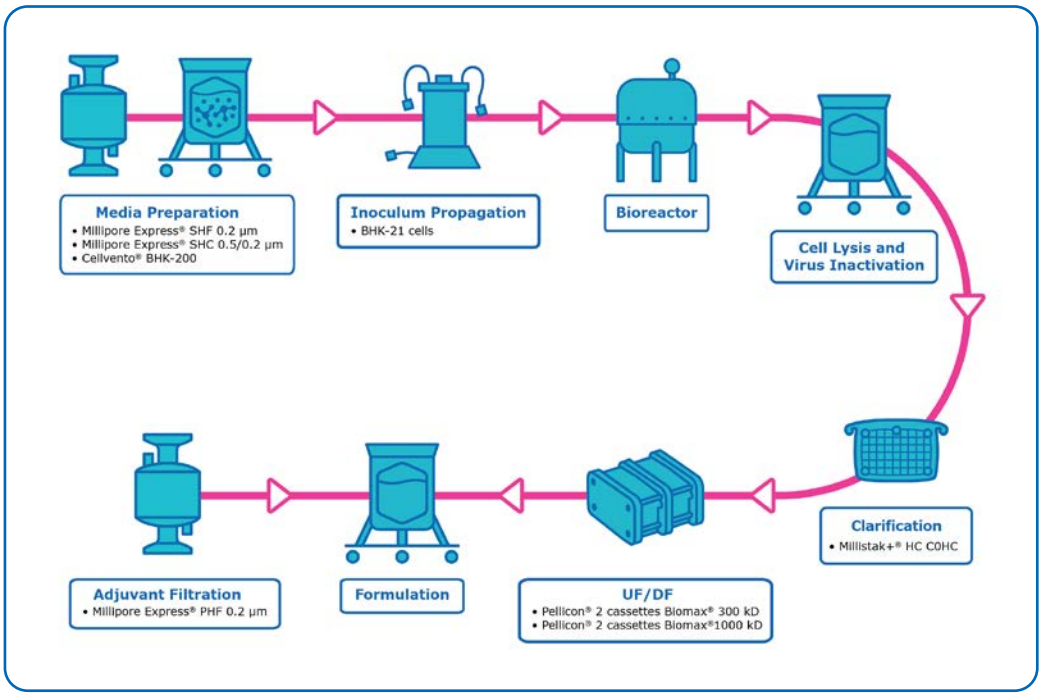

Figure 8. FMD vaccine production process

Considering the strategy to limit the impact of FMD through vaccination, and to ensure sufficient supply of vaccines, a partnership between manufacturers and process solution providers is key to enable a fast and efficient journey to commercialization. This collaboration has here enabled the implementation of an optimized and scalable production platform; starting from the upstream with the use of a recent cell culture media and specifically adapted cells allowing the replication of the FMD virus in a completely serum-free environment. This change strongly reduced the burden on the downstream purification steps and allowed the development of high performing filtration purification strategies to develop a scalable, cost-efficient and regulatory compliant FMD vaccine production. Implementing a more robust process with improved economics is an important step towards enabling greater access to a much-needed vaccine in the animal health market.

Optimizing Downstream Processing for an Inactivated Rabies Vaccine

MilliporeSigma:
Youness Cherradi, Senior Process Development Scientist – EMEA
Youssef Gaabouri, Associate Director Sales, North & West Africa
Anissa Boumlic, PhD, Head of Global Vaccine & Plasma Segments

Institut Pasteur de Tunis:
Héla Kallel, PhD, Head of the Biotechnology Development Group
Khaled Trabelsi, PhD, Senior Engineer, Head of the Biotechnology Development Group

Objective

While rabies is a preventable and curable disease, it continues to have an impact on human and animal health in developing countries. According to the World Health Organization (WHO), infection causes tens of thousands of deaths every year, mainly in Asia and Africa (1). Effective vaccines do exist, but they are not always readily available or accessible to those in need.

Optimizing virus yield during the manufacturing process is essential for development of a low-cost rabies vaccine, which would help ease the burden in low and middle income countries.

We are collaborating with the Institut Pasteur de Tunis, one of the main research centers in Tunisia with vaccine manufacturing capabilities, to optimize their process for manufacturing a rabies vaccine. The overall goal is to establish a rapid, scalable and GMP compliant process for more cost-effective production and increased yield. This white paper describes the improvements made to the downstream portion of the process, specifically, use of single-use technologies and novel techniques for clarification and ultrafiltration/diafiltration.

Summary of Results

- The combination of Millistak+® HC C0HC depth filters and a Polysep™ prefilter used prior to tangential flow filtration improved overall yield of the clarification step from approximately 50% to more than 90%
- High loading achieved on the clarification step enabled cost-efficient and low footprint scale-up
- Pellicon® tangential flow filters allowed concentration and diafiltration of the rabies viral vaccine with minimal loss

Current Process Challenges

The upstream portion of the process starts with production of virus in Vero cells grown on microcarriers in perfusion mode. The upstream process had been optimized in a bench-top bioreactor and then further scaled to a 20L bioreactor (12L working volume) with a maximal cell viability of 81% and a cell density that can reach 9.10^6 cells/mL (2).

Prior to optimizing the downstream process, viral harvests obtained through the culture were clarified using an 8 µm cellulosic membrane followed by a 0.45 µm PVDF membrane (**Figure 1**). Inactivation using β-propiolactone (BPL) and sucrose stabilization was performed prior to filtration on the 0.45 µm membrane. Zonal centrifugation in a sucrose density gradient was then used for purification.

Yield losses observed with an older, disc-style 0.45 µm membrane filter that was not scalable drove interest in integration of new clarification technologies to minimize this loss, enable scaling and modernize the process. The downstream process was further optimized downstream with the addition of tangential flow filtration (TFF) which allows concentration of the filtrate and buffer exchange prior to the chromatography step.

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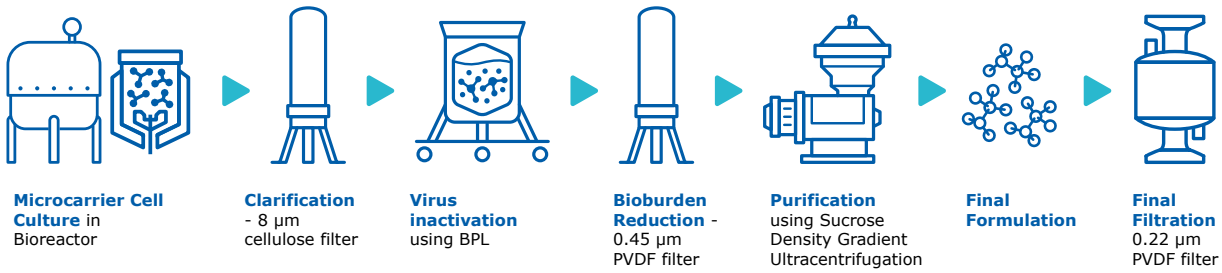


Figure 1. Initial rabies vaccine production process.

Table 1 summarizes the depth filters evaluated for clarification, prefilters and TFF membranes that were evaluated for use in downstream processing of the rabies vaccine. Millistak+® CE and DE media deliver The Clarisolve® filter is made of polypropylene and is not charged. The prefilters are 0.5 µm rated with different chemistries; Polysep™ is a mix of cellulose and glass fiber, Durapore® is polyvinylidene fluoride (PVDF) and Millistak+® is cellulose.

Prefilter	Details	Membrane Area (cm^2)	Catalog Number	
Polysep™ 1.0/0.5 µm	Prefilter: BGF Filter: MCE	17.7	SGW6A47FF3	
Milligard® 1.2/0.5 µm	MCE	17.7	SWSCA47FF3	
Durapore® 0.45 µm	PVDF	3.5	SPHLA25NB6	
Depth Filter	**Details**	**Membrane Area (cm^2)**	**Catalog Number**	
Millistak+® D0HC	25CE + 40DE	23	MD0HC23CL3	
Millistak+® C0HC	30DE + 60DE	23	MC0HC23CL3	
Clarisolve® 60HX	Polypropylene	23	CS60HX01L3	
TFF Device	**Membrane**	**Screen**	**Catalog Number**	**Area (m^2)**
Pellicon® 2 cassette	Biomax® 100 kDa	V	P2B100V01	0.1
Pellicon® 2 cassette	Biomax® 300 kDa	C	P2B100C01	0.1

Table 1. Specifications of the depth filters, pre-filters and TFF membranes evaluated for optimizing downstream processing.

Optimizing the Downstream Process: Clarification

The bioreactor used in the original process included a stainless-steel spin filter which was used to remove microcarriers from the feed stream. In parallel with this study to optimize the downstream process, a Clarisolve® 60HX depth filter was evaluated for the removal of microcarriers as a potential replacement of the currently used stainless steel spin filter (data not shown). While the concentration of microcarriers used in culture was approximately 3 g/L, the ability of Clarisolve® depth filter to remove carriers at a concentration up to 6 g/L was demonstrated. Additional characteristics of the feed streams are detailed in **Table 2**.

Product of Interest	Rabies Vaccine
Size (nm)	180 nm long and 75 nm wide
Product concentration (mg/L)	2.27
Other components	VP-SFM medium + Sucrose 5%
Cell line type	Vero. Cytodex 1 microcarrier (3g/L)
Cell density (x10⁶ cells/mL)	3 to 4
Cell viability (%)	<30%
Fermentation type	Perfusion
pH	7.4
Stability	Several days at +4° C
Phase	Preclinical

Table 2. Characteristics of the virus production feed stream.

The impact of different hydraulic conditions in combination with Millistak+® D0HC and C0HC depth filters on yield are shown in **Table 3**. When the flux was increased across the C0HC filter (comparing trials Millistak+® C0HC #1 and Millistak+® C0HC #2), there was an increase in yield from 52% to 92%. Addition of a buffer flush step to trials Millistak+® D0HC and Millistak+® C0HC improved recovery of the virus (**Figure 2**).

Depth Filter	Trial Flux (LHM)	Trial Loading (L/m²)	Trial Endpoint Pressure (psi)	Initial Turbidity (NTU)	Filtrate Pool Turbidity (NTU)	Yield (%)
Millistak+® C0HC #1	103.2	369.6	0	1.31	0.7	52
Millistak+® C0HC #2	181.2	347.8	0	5.45	1.66	92

Table 3. Summary of results using Millistak+® C0HC depth filters.

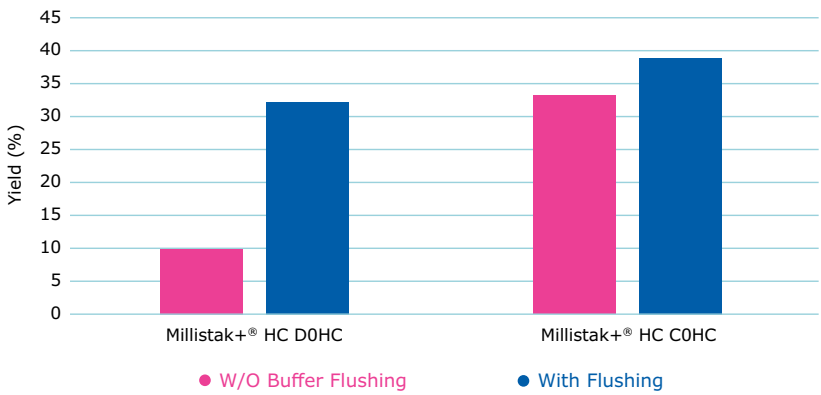


Figure 2. Use of a buffer flush step improved recovery of the virus.

Following these initial results, a larger feed stream volume was clarified using the Millistak+® C0HC filter and Polysep II™ 1.0/0.5 µm pre-filter. The ability to filter a larger volume using the same filter footprint provides the opportunity to improve process economics.

This trial was performed with the same feed stream as used for C0HC #3 (**Table 3**) and demonstrated the impact of loading on the recovery. Two hold-up volumes of phosphate buffered saline (PBS) buffer were added following depth filtration. The final yield could be significantly improved from 39% to 91% when the filtration loading was increased from 400L/m^2 to more than 1700L/m2 (**Table 4**). This result can be explained by saturation of non-specific adsorption sites of the filter.

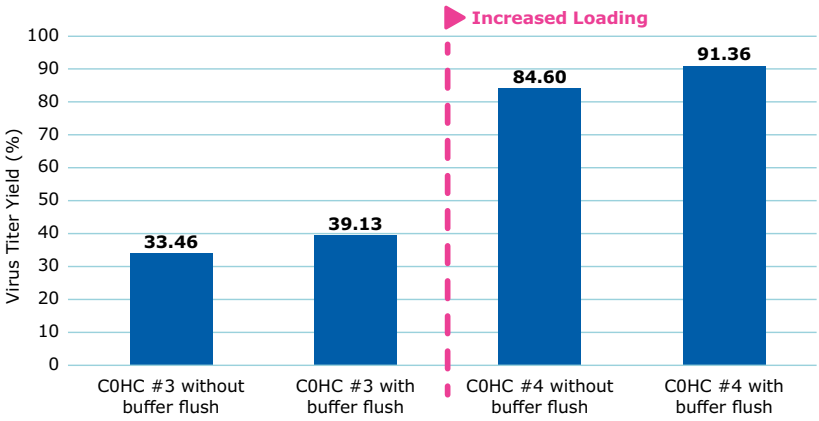


Figure 3. Use of a buffer flush step increased flux.

Depth Filter	Trial Flux (LHM)	Trial Loading (L/m^2)	Trial Endpoint Pressure (psi)	Initial Turbidity (NTU)	Filtrate Pool Turbidity (NTU)	Yield (%)
Millistak+® C0HC #4	134.1	1739.1	0	5.3	~2	91.4
Polysep® II 1.0/0.5 µm filter	166.6	434.8	0			

Table 4. Results of loading optimization study.

Optimizing the Downstream Process: Concentration/Diafiltration

Pellicon® 2 TFF devices with 100 kDa and 300 kDa BioMax® membranes were tested at 4 L/min/ m². The optimization curves (data not shown) indicated an optimal transmembrane pressure (TMP) of 20 and 30 psi respectively. Both membranes provided similar yields as shown in **Table 5**.

TFF Membrane	CF/DF	Trial Loading (L/m^2)	TMP (psi)	Conc. Flux (LMH)	Diaf. Flux (LMH)	Yield (%)
300 kDa	13.1/5.9	18.47	30	185	142	85.0
100 kDa #1	11.7/5.4	18.3	20	143	123	88.4
100 kDa #2	11.7/5.1	58.95	18	130	93	79.1

Table 5. Comparison of 300 kDa and 100 kDa membranes for concentration/diafiltration.

Figures 4 and 5 show the impact of the volumetric concentration factor (VCF) and diavolumes on performance of the 100 kDa BioMax® TFF membranes, respectively. In both cases, permeate flux, TMP and Pin (Inlet Pressure) remained within the desired ranges.

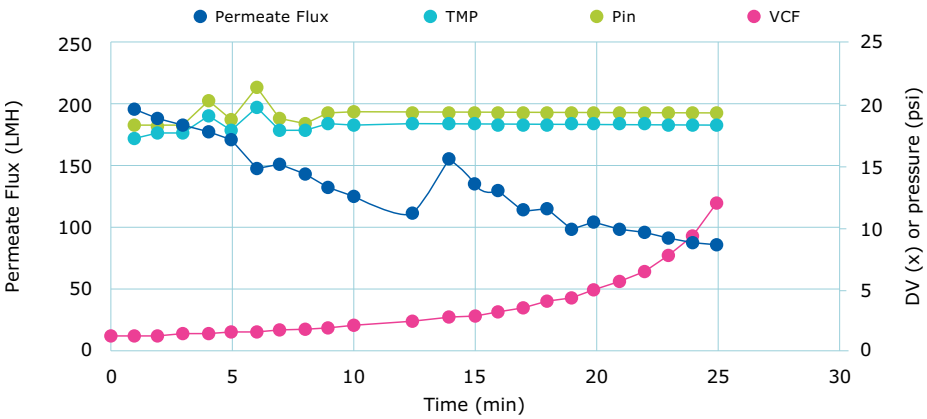


Figure 4. Impact of VCF on 100 kDa BioMax® TFF membrane operating parameters.

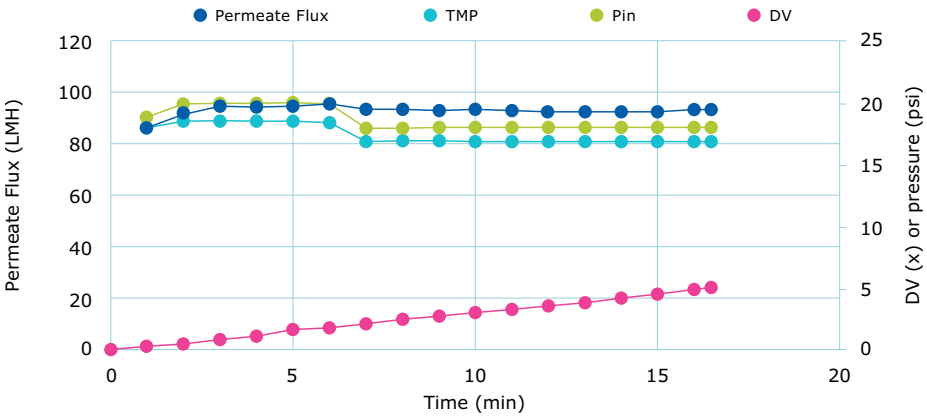


Figure 5. Impact of diavolumes on 100 kDa BioMax® TFF membrane operating parameters.

Samples from permeate of the ultrafiltration and diafiltration steps were analyzed by SDS-PAGE (**Figure 6**). No bands were detected indicating that there was no virus present in the permeate samples; the amount was also estimated by ELISA to be 0 µg/mL (data not shown).

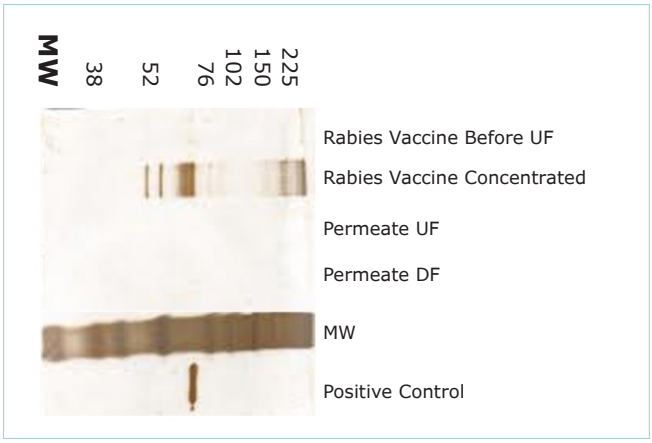


Figure 6. Silver staining analysis of samples following ultrafiltration and diafiltration.

Conclusion

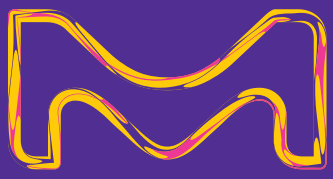
The need for a robust, cost-effective process for the manufacture of a rabies vaccine is clear.

In this study, we collaborated with the Institut Pasteur de Tunis to optimize the clarification step and integrate TFF into the downstream process of their virus manufacturing process. The combination of Millistak+® HC C0HC depth filters with a Polysep™ II prefilter resulted in a significant improvement to the overall yield of the clarification step. Yield was increased with higher loading on the filter and addition of a post-filtration buffer flush. The high loading achieved on the clarification step enables a more cost-efficient and smaller footprint scale-up – essential for the success of rabies vaccine manufacturing. In addition, Pellicon® 2 100 kDa and 300 kDa filters performed with no noticeable issue, allowing the concentration and diafiltration of the rabies viral vaccine with minimal loss.

The potential benefits incorporating this updated process include a significantly increased yield and loading in robust clarification step leading to a lower footprint and lower cost of goods, a bioburden reduction step: changing an outdated disc-format with limited loading resulting with yield and long filtration time to scalable and high performing capsule format, and a reproducible, scalable and easy-to-operate process (TFF versus Ultracentrifugation).

References

1. https://www.who.int/news-room/fact-sheets/detail/rabies
2. Trabelsi et al. 2005; 2006



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