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White Paper

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.

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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.

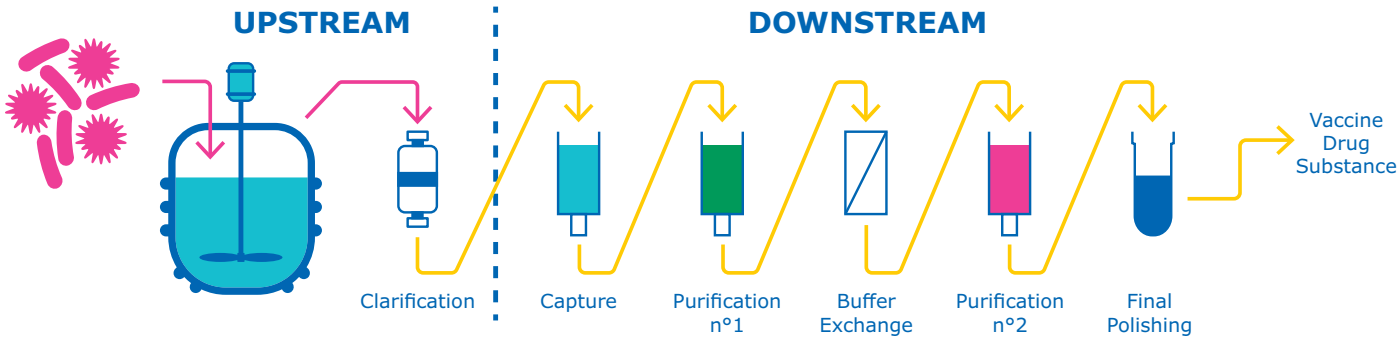


Figure 1. Current situation: multiple steps process = time and water consuming, expensive and limited yields

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

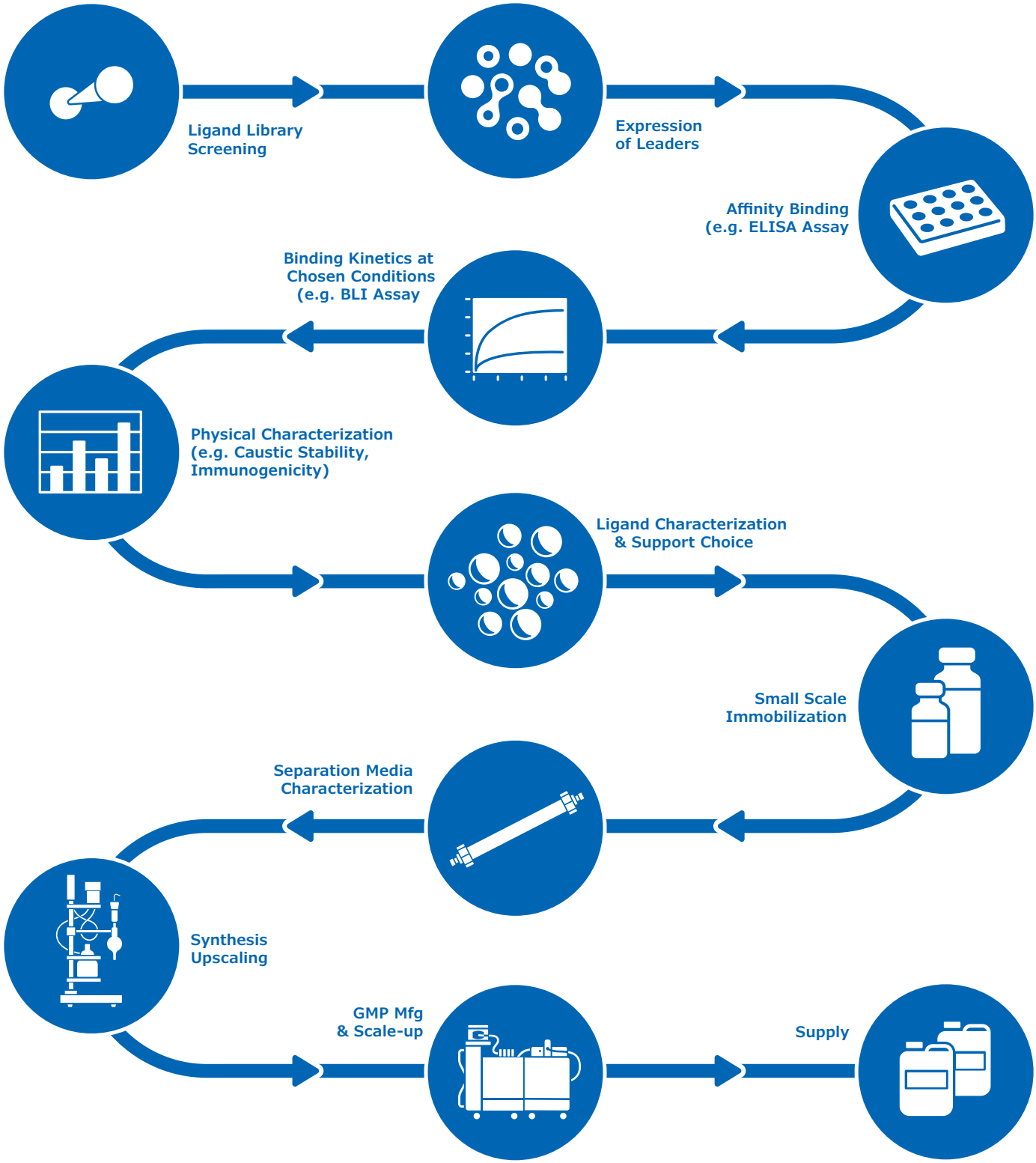
Collectively, these efforts are expected to enable state-of-the-art, robust vaccine-manufacturing systems that can make localized production opportunities more widely available — and thus lifesaving vaccines more affordable — than ever before.

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 KGgA (Darmstadt, Germany), and its Life Sciences 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 (Compenhagen, 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.

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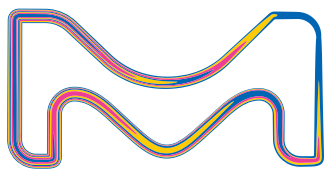

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