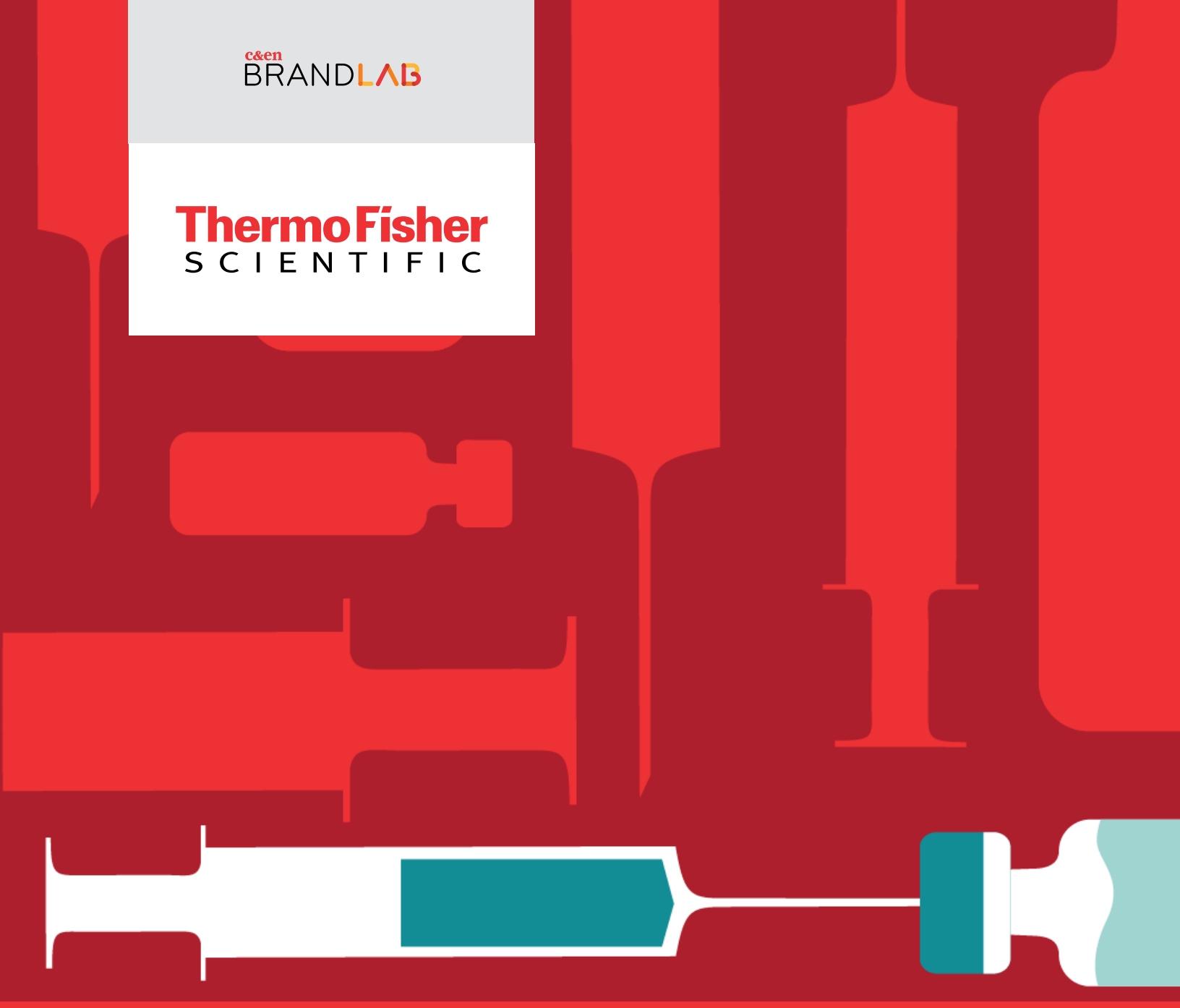
c&en BRANDLAB

ThermoFisher SCIENTIFIC



MANAGING PURITY IN VACCINES:

How advanced purification technologies are speeding the hunt for new protections against disease

Table of contents

Introduction

Smallpox was the first infectious disease for which an effective vaccine was developed. In the late 1700s, British doctor Edward Jenner devised a method to inoculate people with cowpox pus to give them immunity against smallpox, a much more deadly disease. Problems translating this concept into a process suitable for large-scale manufacture slowed the vaccine's distribution for many years, but its use eventually led to the declaration in 1980 that smallpox had been eradicated.

For many vaccines, developing consistent and efficient manufacturing processes is a major hurdle on the path to clinical trials and—after they're approved—to getting them into the arms of all who need them.[1] The active ingredients in vaccines can be antigens in the form of either a whole bacteria or virus, or fragments of them (such as proteins or sugars). Some newer types of vaccines include genetic instructions for bodies to make antigenic fragments. Because these vaccine components are grown in cells, they must be separated out of a complex biological soup before they can be turned into pharmaceutical products.

Purification is challenging and typically the most laborious and expensive stage of vaccine manufacture. The endeavor typically requires a long sequence of chromatography steps that use differences in size, charge, and hydrophobicity to separate biomolecules. Each extra step comes with costs in the form of lost product and time. State-of-the-art purification technologies like affinity chromatography have the potential to streamline vaccine purifications and thus help accelerate vaccine development and manufacture.

Affinity advantages

Affinity chromatography relies on differences in affinity between the chromatography resin and the biomolecules in the mixture to accomplish separation.

A wide range of affinity resins are available, including those that use antibodies or heavy metal ions to selectively bind target biomolecules. The unwanted product- and process related impurities in the mixture pass through the column before the conditions are changed to release the target biomolecule. For example, resins containing a protein A ligand, such as Thermo Fisher Scientific's MabCaptureC™ affinity matrix are used extensively for capturing monoclonal antibodies.

Affinity resins can also be used to purify messenger RNA (mRNA) vaccines and other mRNA therapeutics. The Moderna and Pfizer-BioNTech COVID-19 vaccines use mRNA technology. Several mRNA vaccines against other diseases, including Zika virus and rabies, are in development. In early 2020, Thermo Fisher Scientific launched an affinity resin for mRNA purification.[2] POROS™ Oligo (dT)25 beads are coated with deoxythymine (dT) strands that capture mRNA's poly-A tail.

An emerging type of vaccine with unique purification challenges are the virus-like particle (VLP) vaccines, already in use for hepatitis B and the human papillomavirus, which can cause cervical cancer. VLP vaccines grown in baculovirus-based insect systems require a purification strategy that thoroughly removes baculovirus particles and fragments. One approach is to use affinity resins that capture specific impurities, such as those from baculovirus-based insect systems, while target biomolecules pass through the column. Thermo Fisher's POROS™ CaptureSelect™ BacuClear performs in this way[3] and is specifically designed for use with VLP vaccines grown in baculovirus-based insect systems.

Tags are it

Use of the type of affinity resins discussed in the previous section is limited to target biomolecules, or impurities, for which suitable chromatography resins can be developed. Affinity tags are opening up affinity chromatography to a much broader selection of target biomolecules. These tags are short peptide sequences attached to target biomolecules via genetic engineering.

While research and development teams have been using affinity tags in the lab for around 30 years, vaccine manufacturers are just beginning to. Their hesitation most likely has two causes: One, leaving a tag on a vaccine component could open up the potential

discussion for unwanted side effects caused by the body's immune system generating an immune response to the tag. Two, cleaving the tag off introduces an extra manufacturing step.

In at least some instances, these concerns seem to be proving unwarranted. The Jenner Institute at the University of Oxford recently received approval to leave a very short affinity tag on malaria vaccine candidates during clinical trials.[4,5] The Oxford team is using the C-tag that is just four amino acids long—too short for the body to generate an immune response against. So far, six malaria vaccines containing a C-tag and purified with Thermo Fisher's CaptureSelect™ C-tagXL affinity matrix have been green-lit for clinical trials. The Oxford team believes the US Food and Drug Administration and other global regulatory bodies will also permit the C-tag to be left on their vaccines once they are fully approved.[4]

In the coming years, affinity chromatography will find growing use in the manufacture of vaccines and will enable producers to make these lifesaving tools more affordably and at scale. This e-book will explore using affinity chromatography to help accelerate vaccine development (chapter 1), to manufacture mRNA vaccines (chapter 2) and VLP vaccines (chapter 3), and to develop malaria vaccines (chapters 4 and 5).

REFERENCES

1. C&EN BrandLab, *Purification and Quality Control Solutions to Accelerate Vaccine Development,* Thermo Fischer Scientific.
2. C&EN BrandLab, *Get in the mRNA Vaccine Race with Affinity Chromatography,* Thermo Fisher Scientific.
3. C&EN BrandLab, *Addressing Challenges Across the Virus Like Particle Manufacturing Workflow,* Thermo Fisher Scientific.
4. C&EN BrandLab, *Affinity Tags Head Toward the Vaccination Clinic,* Thermo Fisher Scientific.
5. C&EN BrandLab, *How Affinity Tags Are Speeding Up Malaria Vaccine Development,* Thermo Fisher Scientific.

CHAPTER 1

Purification and quality control solutions to accelerate vaccine development

Vaccines are a critical form of defense against disease. Yet in 2018, 20 million children worldwide did not receive basic vaccines.[1] Sirat Sikka, a field applications scientist at Thermo Fisher Scientific, says this is due in part to the challenge of delivering vaccines to rural or remote locations but also reflects the difficulty of manufacturing the number of doses required to meet global demand in the first place.

The need for rapid vaccine development is most vividly demonstrated by the COVID-19 pandemic, caused by the SARS-CoV-2 virus. Vaccines will be a valuable tool in combating this disease as well as new diseases that arise in the future. Disseminating novel vaccine manufacturing processes becomes as crucial as producing and distributing ample supplies of doses. “The COVID-19 pandemic has been a wake-up call and shows that there is a compelling need to be prepared for new diseases,” Sikka says. “We need to be able to rapidly manufacture large numbers of safe and effective vaccine doses for use worldwide.”

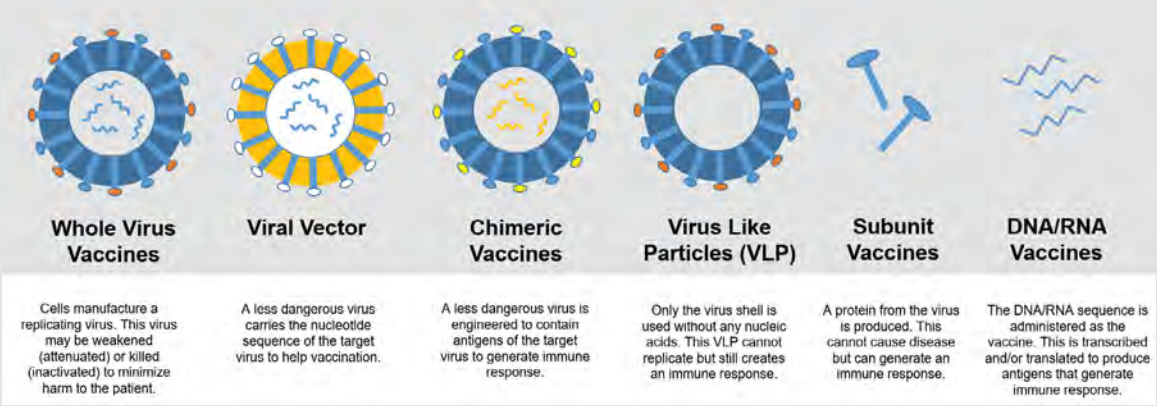


Figure 1: Vaccines vary in composition but include at least one molecular antigen or nucleic acid sequence that encodes an antigen capable of generating a specific immune response. Each vaccine type presents different manufacturing and processing challenges. *Source: Thermo Fisher Scientific*

We need to be able to rapidly manufacture large numbers of safe and effective vaccine doses for use worldwide. — Sirat Sikka, Thermo Fisher Scientific

Traditional vaccines consist of whole nonviable pathogens or pieces of those pathogens that spur an immune response. New types of vaccines have emerged in the past decade, and vaccine development and manufacturing pipelines now include viral vectors, messenger RNA (mRNA), and DNA (Figure 1).

Establishing a manufacturing process for each new vaccine type remains a bottleneck, however. Each type of vaccine comes with its own manufacturing and processing challenges and requires extensive development and analytical testing, Sikka says. This could increase timelines and costs associated with production while potentially delaying clinical trials.

When considering good manufacturing practices for new vaccines, companies need to ensure that the synthesis and processing methods in place consistently produce the same product and that the product meets specific quality standards. Innovative solutions for both biomolecule purification and quality assessment are critical to accelerate vaccine development, approval, and deployment.

Chromatography in vaccine purification

Vaccines may consist of a whole virus, DNA, mRNA, or a protein, but vaccines all carry at least one molecular antigen or a nucleic acid sequence that encodes an antigen. This antigen, which typically is a protein or polysaccharide, induces the specific and targeted immune response against a particular infectious agent. A key step in vaccine production is a high-quality purification of the molecule that ultimately triggers an immune response. Purification typically involves chromatographic steps that exploit specific biochemical and biophysical properties of the vaccine components.

While the overall vaccine production and quality assessment process may look similar between vaccines with different molecular components, each step has to be optimized and tailored to each vaccine's bioactive compound while maintaining product quality, according to Sikka. "Innovative chromatography solutions are important to facilitate

shorter processing times, higher productivity, and reduced cost during the development and manufacture of vaccines," she says.

Chromatographic purification can be broadly divided into two categories: affinity purification and nonaffinity purification. In affinity purification, the ligand specifically isolates the molecule of interest away from the complex starting material based on macromolecular binding interactions between the biomolecule and the ligand. Nonaffinity purification relies on the main properties of a biomolecule: size, charge, and hydrophobicity. Although not as specific as affinity purification, nonaffinity chromatography is also a critical part of vaccine production.

Improved affinity resins allow for efficient purification

In affinity chromatography, the target molecules may be modified to include a short tag that adheres to complementary affinity resins. Alternatively, the affinity ligand on the resin could be an antibody or other molecule that is specific to the molecule of interest without the need for a tag.

Affinity resins can help researchers quickly produce vaccine candidates for screening, which in turn can accelerate the development and clinical testing of promising new vaccines. "Any process that involves specific capture of the molecule of interest while washing away the impurities is going to drive the efficiency of the overall purification process," says Mike Brewer, global principal consultant regulatory of Thermo Fisher Scientific's bioproduction division. "Affinity chromatography greatly reduces the complexity of the sample that's going into the next step, so it can shorten overall process time and cost."

The specificity of affinity purification is particularly helpful in the purification of biomolecules, which are costly and time consuming to produce and tend to be susceptible to degradation over time. Without affinity purification, Sikka says, scientists would need to implement multiple nonaffinity chromatographic steps, which could result in product loss at every step. A purification strategy that relies exclusively on nonaffinity chromatography may also lengthen overall processing time and lead to product stability concerns in addition to yield loss.

Affinity tags for protein purification are typically short peptides added to either the N- or C-terminal of a recombinant protein. These tags can allow researchers to use established affinity ligands to purify their product away from untagged contaminants.

Rebecca Ashfield, a senior project manager at the Jenner Institute, part of Oxford University in the U.K., uses affinity resins to purify vaccine components. She and colleagues employ a C-terminal tag, called C-tag, that comprises four amino acids. This tag binds specifically to a camelid antibody fragment custom built to capture C-tag sequences, which can be coupled to a resin for affinity purification. This technology is used in Thermo Fisher Scientific's CaptureSelect™ suite of affinity products (Figure 2).

This type of affinity purification is efficient, Ashfield says, adding that it is particularly useful if researchers want to purify several candidates to screen. "With the C-tag and CaptureSelect resins, we can cheaply produce these vaccine candidates to identify the optimal vaccines to take forward to further clinical trials," she says. "The process is efficient, quick, reliable, and gives you a high-purity product."

Not all affinity resins are created equal, however, and some work better for vaccine development than others. For example, Ashfield says, she and colleagues were working on developing a recombinant protein vaccine against malaria, a debilitating and potentially deadly infection caused by *Plasmodium* parasites.[2] The researchers initially tried to purify the protein component of the vaccine using a His tag, which is a string of histidine residues that binds to a nickel-based affinity resin.

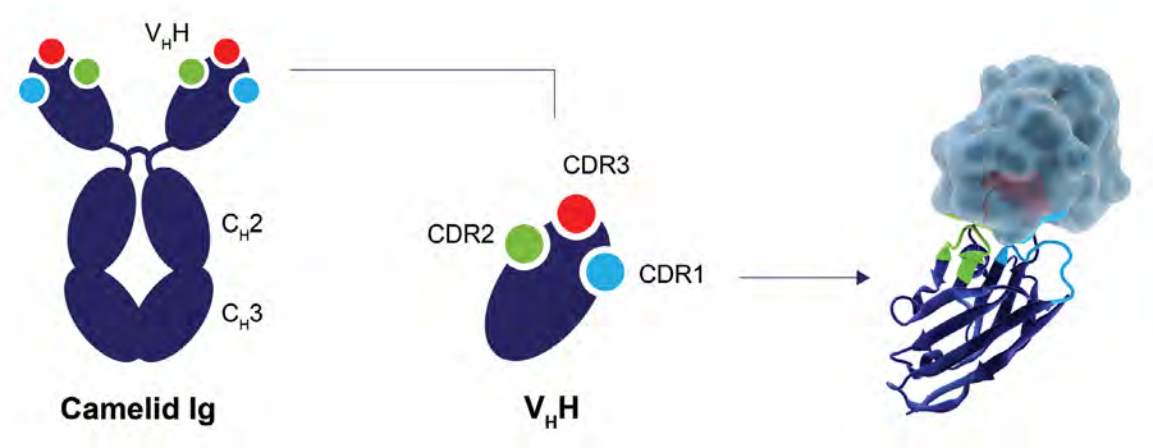


Figure 2: The CaptureSelect affinity ligands are derived from camelid immunoglobulin (Ig) antibodies that have been pared down to the N-terminal domain (V_HH fragment), including just the variable antigen-binding regions, also called complementarity determining regions (CDR), (shown in green, blue, and red). The antigen-binding region of the affinity ligand can be tuned to specifically recognize and capture target molecules (light blue), including known protein tags and untagged proteins. *Source: Thermo Fisher Scientific*

The researchers were having issues with product yield and quality, however, which is not uncommon when developing a new purification process. The Oxford team decided to switch to the C-tag and use the associated CaptureSelect resin during the affinity step.[2] "After switching, we got a massive increase in yield and an increase in purity," she says.

The C-tag is also very short and can be left on the protein without adverse effects. Ashfield says this is a big advantage over other affinity tags that may be longer and thust not acceptable for inclusion in a final licensed product, such as His tag.

Not all vaccine biomolecules are amendable to tagging, possibly because the tag may adversely affect their structure or function. To this end, the same affinity technology used to capture C-tagged proteins is also used to capture untagged ones. These CaptureSelect affinity ligands can provide tunable selectivity to virtually any target, according to Sikka. This way, the ligand will still specifically capture the target molecule without the need to modify it with a tag.

Nonaffinity chromatography to improve processivity

Nonaffinity purification is critical for vaccine development and production even when there are established affinity ligands for targeted biomolecules. Frank Riske, a senior consultant at BioProcess Technology Consultants, says that even in the most well-established and controlled affinity chromatography steps, low levels of affinity ligands leach off the resin. At least one subsequent nonaffinity purification step is needed to remove these impurities associated with using affinity resins.

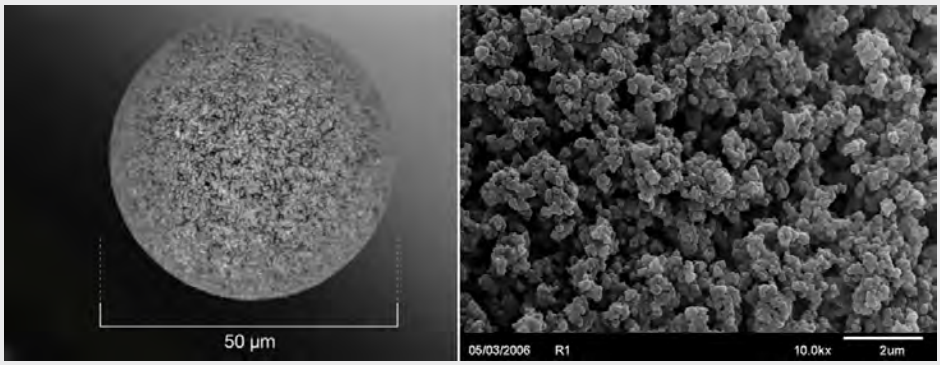


Figure 3: Scanning electron microscope images show a POROS resin bead on the left, with a magnified image on the right showing the large through-pores. These allow for increased surface area and therefore more binding capacity while maintaining the ability to separate out the target molecule from impurities. *Source: Thermo Fisher Scientific*

You can try several different resins and chromatographic steps and then pick the ones that work best for your protein or molecule of interest.

— Frank Riske, BioProcess Technology Consultants

Additionally, some vaccine biomolecules may not be amenable to tag-based affinity approaches or may lack established affinity ligands. The cost of designing new affinity systems may be prohibitive, especially for small-scale or initial screening studies. “It takes time to develop unique affinity ligands for different targets,” Riske says. He adds that developing a specific affinity ligand is challenging and may not be viable for all molecules.

Instead, a series of nonaffinity chromatography steps can purify a vaccine agent, including ion exchange and hydrophobic interaction chromatography. However, there are challenges with nonaffinity approaches, such as the inability to separate out closely related species as well as the time it takes to complete each chromatographic step. High-capacity resins, including the POROS™ nonaffinity resins developed by Thermo Fisher Scientific, can help overcome these challenges (Figure 3).

POROS resins are rigid beads with large pores, which offers an increased surface area. The increased surface area and large through-pores enable more interaction between the target molecules and the resin ligand, meaning that capacity can be increased and more target molecules can be loaded all at one time. This increased capacity can allow for reduced column sizes while still providing sufficient separation of target and nontarget molecules.

“The POROS nonaffinity resins, for example, are a toolbox you can use to develop a purification process,” says Riske at BioProcess. “You can try several different resins and chromatographic steps and then pick the ones that work best for your protein or molecule of interest.”

Accelerating quality control assessment

In vaccine production, product quality needs to be assessed throughout the purification and manufacturing process to ensure that the product meets safety, purity, and regulatory standards.

Rapid detection of contaminants

Quality control (QC) tests typically start before the purification process even begins, Brewer says. Many vaccines are produced using biological systems, including mammalian cell or bacterial cultures, that must be free of contaminants such as viruses and harmful bacteria like mycoplasma.

Mycoplasma are considered the simplest form of bacteria. They are characterized by their small size and lack of a bacterial cell wall. They can infect the mammalian cell cultures commonly used in vaccine production and drastically alter cell characteristics and negatively impact critical quality attributes of both the manufacturing process and final product.

Mammalian cell cultures used to produce therapeutics and vaccines are required to be tested for and free of mycoplasma. The traditional test is culture based, has a time to results of 28 days, and is typically done by specialty testing labs. Between the time it takes to ship samples, get to the front of the testing queue, and actually do the test, mycoplasma detection can cause a significantly delay in the release and distribution of a batch of biotherapeutics or vaccines. An alternative to the time-consuming culture-based test for mycoplasma is to use rapid, quantitative polymerase chain reaction (PCR) testing. Following validation, regulatory review, and acceptance, Thermo Fisher Scientific's MycoSEQ™ Mycoplasma Detection Kit, based on quantitative PCR, is now used by multiple manufacturers globally as an alternative to the 28-day test for mycoplasma. The application of MycoSEQ can reduce the time to result to as little as 5 hours.

Florian Durst, a field application specialist of the pharma analytics group at Thermo Fisher Scientific, says one of the benefits of rapid test results is being able to test at multiple steps of the cell culture process, including before the cell harvest. "You don't want to make it far down the process and then have to throw away thousands of dollars of time and effort because of mycoplasma contamination," he says. "The rapid assay is a big benefit in terms of risk mitigation."

Analysis of impurities

Vaccine manufacturing cell cultures are complex mixtures that include host cell and process impurities. These must be removed during purification to levels that comply with regulatory guidelines, and manufacturers must perform a battery of analytical tests to demonstrate the quality, consistency, purity, potency, and safety of products produced in cell cultures. One of these tests is quantitation of residual host cell DNA, which must be performed during purification and as the product nears its final dosage form, to ensure that levels are in line with regulatory guidelines.

When developing a biomanufacturing process for a vaccine component, researchers often conduct rigorous studies for purification process characterization in order to demonstrate the capability of the process to reduce or remove impurities, including host cell DNA. Rapid yet sensitive tests can help accelerate process development by enabling accurate testing at every point in the process, ensuring the purity of the product at each step.

The resDNASEQ™ portfolio of residual host cell DNA quantitation assays, developed by Thermo Fisher Scientific, offers a strategy for rapidly quantifying host cell DNA impurities (Figure 4). The kits use quantitative PCR to specifically and accurately measure host cell DNA from cell lines typically used as expression systems for vaccine components. Durst says it takes about 5 h to get results, which helps researchers quickly decide if the product is sufficiently pure to be released to the next step.

Another source of impurities in affinity chromatography is the resin itself. It's not uncommon for small quantities of the affinity ligand to leach off the column and be carried over into subsequent purification steps. Ashfield says it's easy to check for CaptureSelect affinity ligands in subsequent steps and the final product using a kit that detects the camelid antibody fragment.

"This helps ensure there aren't any process-related impurities associated with the affinity purification and that our product is highly pure," Ashfield says. "In our hands, this approach has been broadly applicable and has worked on every vaccine candidate we've tried."

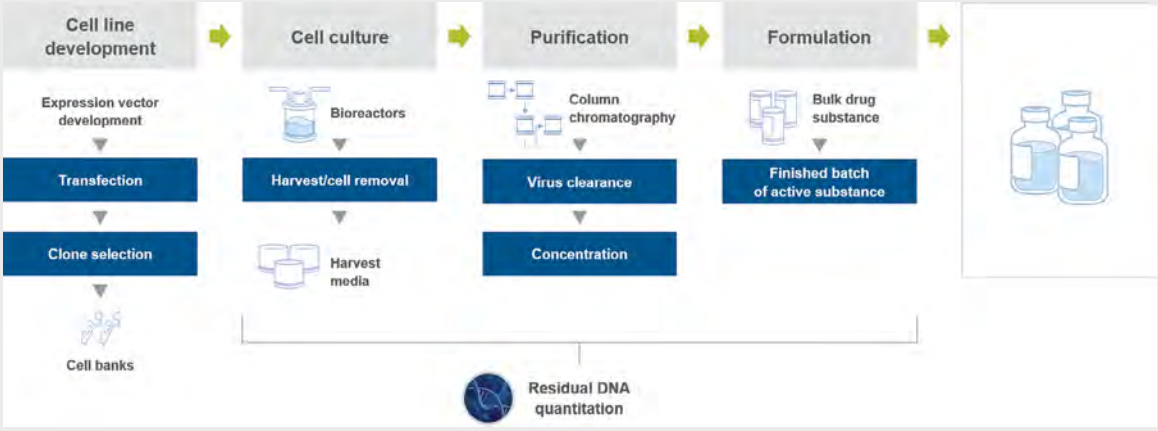


Figure 4: Vaccines need to be tested for residual DNA impurities in the final dosage form, while such testing during manufacturing can help optimize and monitor the process. Quick, reliable assays for residual host DNA can help speed up development of vaccine manufacturing processes, helping finished batches get into the clinic sooner. *Source: Thermo Fisher Scientific*

QC assessments improve process design

Durst says quick quality assessments like the resDNASEQ Human Residual DNA Quantitation Kit can do more than ensure that the product is pure and meets regulatory standards. Instead of using quality tests only as a last step after the biomanufacturing process has been established, he advocates including more QC tests to improve the process as a whole.

QC assessments can "help you assess your manufacturing process and make improvements," Durst says. By having contamination and product purity data at various steps, he says, researchers can improve process development.

In fact, QC tests and assessments can make or break vaccine development. "Fast and accurate tests are of the utmost importance because they help get the product to the finish line and possibly to patients faster," Brewer says. "There's a huge advantage to tests that provide the same assurance of quality and safety but offer the results on a quicker time scale."

Rapid tests like the MycoSEQ Mycoplasma Detection Kit have the added benefit of being conducted in-house, thereby eliminating the need to send testing samples to outside specialized facilities. Brewer says the rapid tests help increase efficiency by avoiding the bottlenecks associated with shipping and handling at the contract lab.

The need remains for ample and new vaccines

Vaccines are estimated to prevent at least 20 million cases of disease in the US each year.[4] As demonstrated by the COVID-19 pandemic, novel vaccines will be needed to combat infectious diseases as they arise.

New types of vaccines made from a range of biological components have emerged as potentially lifesaving ways to prevent disease, yet many lack established biomanufacturing pipelines. Improved chromatographic solutions and QC measures can help ensure these vaccines are not held up by bottlenecks in process development and make it to the clinic faster.

The goal is to have a streamlined and "platformable" approach for each vaccine type, Sikka says. With innovative chromatography and QC assessment solutions, she says she hopes that "we can get to that point which will lead to large-scale cost-effective production of high-quality vaccine, with speed."

FOR MORE INFORMATION, watch the webinar "Accelerating vaccine development by innovative purification solutions and state of the art quality Testing" here.

REFERENCES

1. World Health Organization, "20 Million Children Miss Out on Lifesaving Measles, Diphtheria, and Tetanus Vaccines in 2018," news release, July 15, 2019, https://www.who.int/news-room/detail/15-07-2019-20-million-children-miss-out-on-lifesaving-measles-diphtheria-and-tetanus-vaccines-in-2018.

2. Jing Jin et al., "Accelerating the Clinical Development of Protein-Based Vaccines for Malaria by Efficient Purification Using a Four Amino Acid C-Terminal 'C-Tag,'" International Journal of Parasitology 47, no. 7 (June 2017): 435–46, http://doi.org/10.1016/j.ijpara.2016.12.001.

3. Anthony O. Olarerin-George, John B. Hogenesch, "Assessing the Prevalence of Mycoplasma Contamination in Cell Culture via a Survey of NCBI's RNA-seq Archive," Nucleic Acids Res., 43, no. 5 (March 11, 2015): 2535–42. http://doi.org/10.1093/nar/gkv136.

4. Walter A. Orenstein and Rafi Ahmed, "Simply Put: Vaccination Saves Lives," PNAS, 114 no. 16 (April 18, 2017): 4031–33, http://doi.org/10.1073/pnas.1704507114.

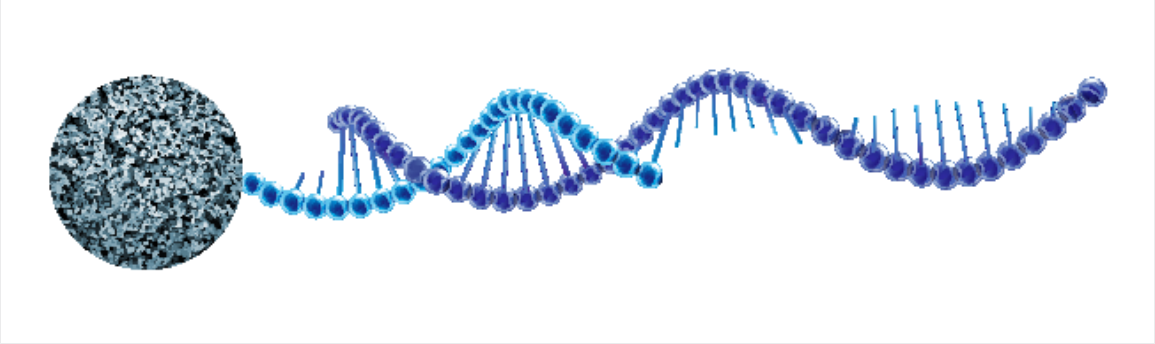
CHAPTER 2

Get in the mRNA vaccine race with affinity purification

On March 16, 2020, the biotechnology company Moderna Therapeutics announced that the first person in a Phase I clinical trial had been dosed with its messenger RNA (mRNA) vaccine against SARS-CoV-2, the virus that causes COVID-19. The company reached this milestone just 63 days after Chinese authorities published the genetic sequence of the virus, SARS-CoV-2.

Developing any new therapy or vaccine from conception to clinical use is a marathon rather than a sprint. But in 2020, the research teams developing mRNA vaccine technology against SARS-CoV-2 have rocketed out of the starting blocks.

With demand for the large-scale production of clinical-grade mRNA suddenly surging, developers need fast, efficient, and highly scalable methods for mRNA purification. The bench-scale mRNA purification methods used until now are becoming a significant bottleneck to large-scale manufacture. Thermo Fisher Scientific has developed a new affinity-based mRNA purification product, Thermo Scientific™ POROS™ Oligo (dT)25 affinity resin, tailor-made for scalability. The mRNA binds selectively to the surface of the Oligo (dT) beads, and any impurities are simply washed away.



Schematic of Thermo Scientific™ POROS™ Oligo (dT)25 affinity resin, consisting of porous polymer beads coated with deoxythymine (dT) strands that can capture mRNA's poly-A tail. *Image credit: Thermo Fisher Scientific*

The speed with which mRNA vaccine candidates can be designed and produced is a key advantage of this new vaccine technology. Traditional protein-based vaccines are inherently time-consuming to produce, even when using the latest cell culture techniques. To date, the shortest vaccine development pathway has been 5 years for the protein-based Ebola vaccine, Ervebo.[1] On April 14, 2020, when two giants of protein-based vaccine production, GlaxoSmithKline and Sanofi, announced that they would collaborate to develop a SARS-CoV-2 vaccine, they forecast that human clinical trials would have to wait until the second half of 2020.

The SARS-CoV-2 crisis could springboard the first mRNA vaccine into widespread use. Even before the disease, mRNA vaccine candidates against pathogens, from Zika virus to rabies, were showing promise in human clinical trials. Aside from vaccines, a variety of mRNA-based therapies are beginning trials for health conditions ranging from common cancers to rare genetic disorders. In May 2019, market analysts at Visiongain forecast that the mRNA vaccines and therapeutics market would grow at a compound annual growth rate of 9.18% between 2019 and 2029, reaching a market value of up to $8.90 billion.[2]

Affinity-based scalable mRNA purification removes one bottleneck to the rapid rollout of mRNA vaccines and treatments for otherwise unstoppable diseases.

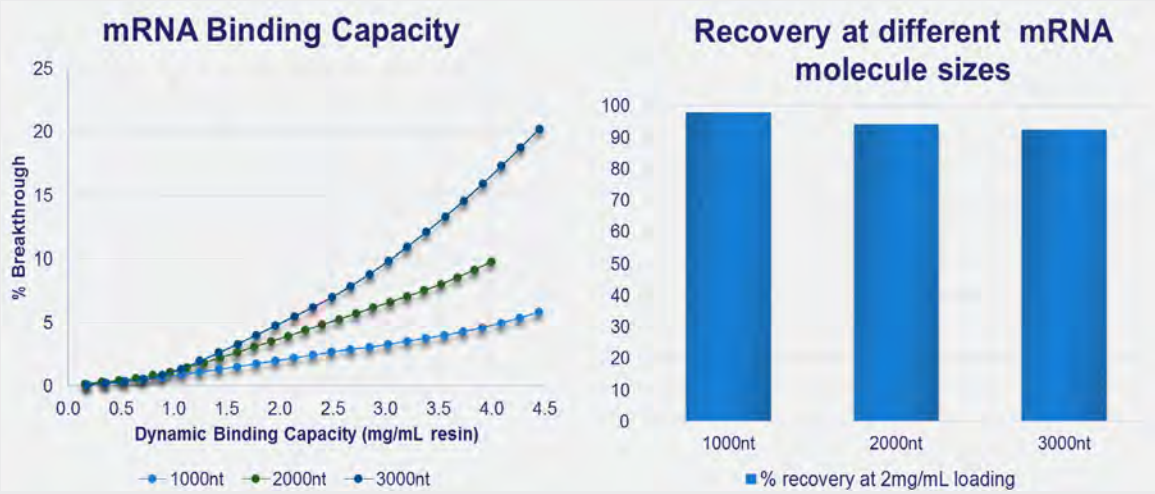
Vaccines

Vaccines based on mRNA are making steady progress through clinical trials toward regulatory approval and clinical use, says Piotr Kowalski, an mRNA researcher at University College Cork. “I think the first clinical applications of mRNA will clearly be vaccinations,” he says. “mRNA is well suited towards that application.”

Particularly in light of the global SARS-CoV-2 research effort, mRNA vaccine production is where the need for high-throughput mRNA purification is likely to become the most acute. With mRNA vaccines and therapeutics, the aim is to piggyback onto the body’s own protein-producing mechanism to gain a health benefit. The natural role of mRNA is to relay protein sequence information from cells’ DNA to the protein-producing ribosomes. When researchers slip synthetic mRNA into cells, the ribosome will produce the corresponding protein.

In the case of vaccines, mRNA is selected that encodes a protein from a virus. Once the injected mRNA is taken up by cells, the body churns out copies of this protein, and the immune system learns to recognize it as an antigen. Many of the mRNA vaccine

candidates for SARS-CoV-2 encode proteins that make up part of the virus's characteristic spiky surface. With a successful vaccine, if a vaccinated person later becomes infected with SARS-CoV-2, the immune system would be trained to recognize and fight it.



Dynamic binding capacity of the resin is influenced by the size of the mRNA construct size (left), while recovery is size independent (right). *Image credit: Thermo Fisher Scientific*

Currently, traditional vaccine makers manufacture the antigen protein itself—a cell-based process that takes months to set up and run. "With mRNA vaccines, the patient's own body becomes the antigen factory," Kowalski says. As mRNA can be rapidly manufactured in a cell-free system, mRNA vaccines are faster to produce than protein vaccines.[3] The switch to affinity purification—already used to purify protein-based biologic drugs at large scale – should further streamline the process.

There are several other reasons mRNA lends itself to vaccination, Kowalski says. Protein-based vaccines generally require a second ingredient, known as an adjuvant or excipient, to boost the immune response and help ensure the antigen generates strong, long-lasting immunity. With mRNA, however, the immune system is inherently on the lookout for it because many viruses are RNA-based. "The mRNA is a natural adjuvant, which helps to boost the immune response," Kowalski says.

The body's virus surveillance systems can be exploited in further ways to boost mRNA vaccine efficacy, says Harry Al-Wassiti, who is developing mRNA-based vaccines and therapeutics at Monash University. He and his colleagues have been researching the

lipid nanoparticles used to encapsulate mRNA prior to injection. “The nanoparticles have two roles: to protect the mRNA and to deliver it to cells,” Al-Wassiti says. For vaccines, immune cells called antigen-presenting cells (APCs) must see the antigen to elicit the immune response. “From the surface, these lipid nanoparticles look almost like a virus, so the APCs take them up,” he says. The Monash team and others have been developing lipid nanoparticles that APCs recognize particularly effectively.

Because of these theoretical advantages, several companies are pursuing mRNA vaccines. “We know we can develop and manufacture them much quicker than conventional vaccines—the question is how well they will work,” Kowalski says. “Everybody is looking for the clinical data, and so far, the data looks promising.” For example, Moderna also has an mRNA vaccine in Phase II clinical trials for cytomegalovirus, a leading cause of birth defects. The company has conducted Phase I trials on eight other mRNA candidate vaccines, including against the H10N8 influenza virus and H7N9 avian flu virus.[4]

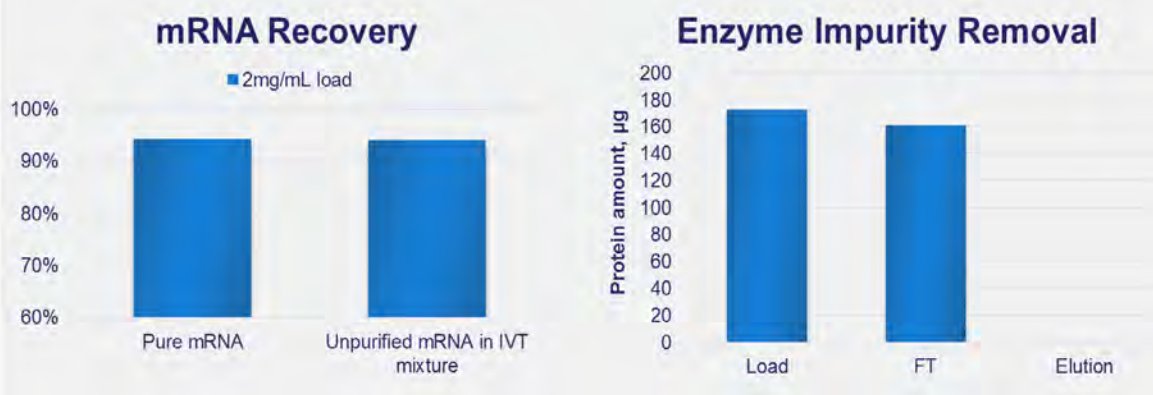
The mRNA company CureVac, based in Tübingen, Germany, has a rabies vaccine in Phase I clinical trials and recently announced that the vaccine elicited a strong immune response with two shots of a microgram mRNA dose. “That’s a really low dose compared to regular vaccines, so there is a lot of promise,” Al-Wassiti says. It’s another shot in the arm for the efficacy of mRNA vaccines. CureVac is also actively working on a SARS-CoV-2 vaccine, as are Al-Wassiti and his colleagues.

Blocking infections isn’t the only application of mRNA-based vaccines, which are being explored and tested for a number of other conditions. Cancer is one of the most actively explored areas. “Instead of a virus, you identify the tumor antigen and try to train the immune system to go after the tumor,” Kowalski explains. The immune system learns to recognize and kill the cancer cells. If approved, a cancer vaccine would require large-scale mRNA production and purification. Both Moderna and CureVac have clinical trials underway for vaccinations against cancer.

Treatments

Vaccines are not the only mRNA-based interventions that could direct the immune system to attack a person’s cancer, Kowalski adds. As a postdoctoral researcher at the Massachusetts Institute of Technology, he used mRNA to generate antibodies as a cancer therapy. Antibody proteins, such as trastusumab (Herceptin), are already used as therapeutics against some cancers. Kowalski and his colleagues demonstrated that there could be health benefits to dosing patients with the trastusumab mRNA and having the body make the antibody.[5] “Injected antibodies are often quickly cleared from the

body," he says. But with mRNA, the body continually produces the protein for a period, somewhat like a slow-release version of the antibody. "We showed we could express the antibody directly in the mouse, to achieve a better pharmacokinetic profile and a better efficacy in terms of treating cancer," Kowalski says.



Unpurified mRNA in an in vitro transcription (IVT) mixture shows similar recovery to loading pure mRNA onto the columns, meaning impurities the in feedstock do not have an impact on final recovery (left). Using the POROS Oligo (dT)25 resin >99% of the protein impurities is removed from the IVT feedstock. Levels of protein in the elution pool are below detection limits. *Image credit: Thermo Fisher Scientific*

Another advantage of mRNA therapeutics compared with proteins is that, from a purification standpoint, all mRNA molecules are essentially identical, Al-Wassiti says. That's because the amino acids in proteins are chemically diverse, while the ribonucleic acids that make up mRNA are relatively similar. "Whereas protein purification really varies depending on the protein sequence, all mRNA looks the same, so the way you purify, it is the same," Al-Wassiti says. It's therefore likely that large-scale mRNA affinity purification protocols pioneered for vaccine development could be readily adopted for mRNA therapeutics.

mRNA also has a number of potential applications in gene therapy. For instance, genetic disorders in which people lack or produce defective copies of a particular protein may be prime targets for mRNA treatments. People with cystic fibrosis, the most common fatal inherited disease in the US, lack a functional copy of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, leading to mucus buildup in the lungs and other organs. By dosing with the relevant mRNA, the body can produce functioning copies of CFTR protein.

Whereas protein purification really varies depending on the protein sequence, all mRNA looks the same, so the way you purify, it is the same. — Harry Al-Wassiti, Monash University

Some of the biggest challenges of mRNA protein replacement therapies concern delivery and safety, Kowalski says. The key is to get the mRNA into the cells that need it, while avoiding other cells where expressing the protein could have a deleterious effect. But certain areas of the body already can be targeted selectively. For example, lipid nanoparticles loaded with therapeutic mRNA can be targeted to the liver, where a range of diseases could be treated. For cystic fibrosis, inhaled formulations designed to deliver mRNA directly to the lungs are being developed.6 Lexington, Massachusetts–based Translate Bio is testing an inhaled mRNA cystic fibrosis therapy in a Phase I/II clinical trial.

Complementing the protein replacement work, mRNA is well suited to knock out specific genes. The therapeutic mRNA can encode the expression system for the gene-editing tool CRISPR-Cas9. “With CRISPR-Cas9 we can also use mRNA to efficiently turn off gene expression,” Kowalski says. Again, delivery is a key challenge and one of the major areas of research focus for his group. Once ready for scale-up, mRNA-based gene-silencing products could benefit from the high-throughput affinity purification protocol pioneered in areas of mRNA research that are a step or two further along the path to clinical use.

Meeting demand

As multiple mRNA drug and vaccine candidates progress from bench-scale research toward clinical use, the demand for larger quantities of clinical-grade mRNA is increasing rapidly. “Therapeutic functional messenger RNAs are a really hot topic and a dramatic driver of growth for us,” says Peter Scheinert, CEO and founder of AmpTec, a Hamburg, Germany–based contract manufacturing company of current good manufacturing practices–grade synthetic nucleic acids for therapeutic and diagnostic applications. “In the past year, we have doubled the number of employees in this area,” he says.

When Thermo Fisher approached AmpTec in 2019 to test a new affinity-based mRNA purification product specifically designed for large-scale applications, the company was very receptive to the idea, Scheinert says. “We were very excited, because it is very

important for us to actively prepare for the increasing requests and demands from the market regarding scale," he says. "mRNA production scales will certainly increase, and we need purification options available that can deal with large scales."

Until now, most manufacturers have used reverse-phase high-performance liquid chromatography (HPLC) to purify mRNA. But that has two major limitations, according to Scheinert. At small scale, HPLC offers very high-resolution separation of components in a reaction mixture. As scale increases, larger column beads are necessary to prevent the pressure from becoming too high, which could compromise separation performance. In addition, scaling up reverse-phase HPLC requires larger volumes of toxic organic solvents, such as acetonitrile, which have negative health and environmental effects.

AmpTec had therefore already ruled out HPLC and was using alternative mRNA purification options, Scheinert says, but the technique is only suitable for purifications of up to 1–2 grams. "For larger scale, there is a lot of hands-on time, which is not suitable for scales of 10 grams, 20 grams, or larger," he adds.

Thermo Fisher customers had been requesting custom solutions for large-scale mRNA purification, according to Scott Zobbi, the firm's business lead for custom POROS chromatography resins. "When three or four people start to ask you the same thing, you realize there's a broader demand," he says. Kelly Flook, its senior product manager for bioprocess purification resins, led the development of an mRNA affinity purification product that would be available to all customers: POROS Oligo (dT)25 affinity resin. AmpTec is among several mRNA manufacturing companies that have helped put the new product through its paces.

Oligo (dT)25 resin leverages the fact that all mRNA molecules, natural and synthetic, feature a poly-A tail, a stability-enhancing chain of adenine nucleotides at one end of the molecule. The product, which exploits complementary base pairing between adenine and thymine to isolate mRNA after synthesis, consists of porous polymer beads coated with deoxythymine (dT) strands that can capture mRNA's poly-A tail.

At the end of an mRNA synthesis, the reaction mixture is combined with a sodium chloride solution, then loaded onto a column filled with Oligo (dT) beads. The sodium ions in the salt solution neutralize the negative charges found along the backbone of the RNA molecules; that allows the poly-A tail to form hydrogen bonds with the dT strands on the beads. The impurities from the reaction mixture are simply washed off the column when flushed with more of the salt solution. When the column is flushed with fresh water, the sodium ions are then washed away. The negative charges on the backbones of the Oligo

(dT) and the poly-A tail repel each other, breaking the base pairing and releasing the now purified mRNA. “Within a couple of column volumes, you will have collected a purified, concentrated solution of your target mRNA,” Flook says.

Part of the product’s appeal is that dA-dT affinity binding is a tried and true method for purifying mRNA samples at bench scale, Scheinert says. The new product transfers the Oligo (dT) coating to a 50 µm polystyrene divinyl benzene cross-linked porous bead. Compared with typical HPLC resins, the bead is large, according to Zobbi. “But because it has inherent porosity, you have an increased surface area,” Zobbi adds, which means greater capacity to bind mRNA. And as it’s a bead-based product, users have the flexibility to pack it into a column of any size. They can tailor the purification step to the scale of the mRNA sample to be purified.

Basing Oligo (dT) on the proved POROS bead technology inspires confidence in the product, says Joseph Barberio, the director of process development at Strand Therapeutics, a seed-stage biotech company developing programmable mRNA therapeutics based in Cambridge, Massachusetts. “POROS resins are proven at scales from benchtop to commercial manufacturing operations,” says Barberio, who has tested the new resin. “Utilizing the same technology from early development through scaled manufacturing is key to the successful tech transfer and execution of a manufacturing campaign.”

Barberio says his experience with Oligo (dT)25 resin has so far been positive. “For so long, the RNA sector has been working with resins that were not made for RNA. It is really great to see a major manufacturer focused on designing products for the mRNA space.”

Scheinert was also gratified by the move. “I was very happy to hear there would be a large-scale option for Oligo (dT) purification,” he says.

With large-scale orders from customers expected soon, AmpTec is prioritizing the switch to POROS Oligo (dT)25 affinity purification, Scheinert says. The company is likely to adopt the product for all mRNA purifications, he adds. “It would make sense to have one

Utilizing the same technology from early development through scaled manufacturing is key to the successful tech transfer and execution of a manufacturing campaign. — Joseph Barberio, Strand Therapeutics

purification method that applies at all scales, in order to have consistent product quality. It is really very flexible, so it would be a good idea to use it exclusively for all mRNA batches—large and small."

The development of mRNA vaccines and therapeutics is a very active space, with progress being made on multiple fronts. The advancements are being further driven by SARS-CoV-2. "There is so much basic research coming out, a lot more open access publishing and data sharing," Kowalski says. "It is an unprecedented crisis, and the response of the scientific community is also unprecedented." Mastering large-scale mRNA purification may be the key to producing a future therapy against SARS-CoV-2 and, with so many mRNA products in development, many other diseases as well.

REFERENCES

1. Tung Thanh Le et al., "The COVID-19 Vaccine Development Landscape," Nat. Rev. Drug Disc. 19, no. 5 (May 2020): 305–06, https://doi:10.1038/d41573-020-00073-5.

2. Visiongain, mRNA Vaccines and Therapeutics Market Forecast 2019-2029, May 2019, https://www.visiongain.com/report/mrna-vaccines-and-therapeutics-market-forecast-2019-2029/.

3. Norbert Pardi et al., "mRNA Vaccines—a New Era in Vaccinology," Nat. Rev. Drug Disc. 17, no. 4 (April 2018): 261–79, https://doi.org/10.1038/nrd.2017.243.

4. R.A. Feldman et al., "mRNA Vaccines against H10N8 and H7N9 Influenza Viruses of Pandemic Potential Are Immunogenic and Well Tolerated in Healthy Adults in Phase 1 Randomized Clinical Trials," Vaccine 37, no. 25 (May 31, 2019): 3326–34, https://doi.org/10.1016/j.vaccine.2019.04.074.

5. Yulia Rybakova et al., "mRNA Delivery for Therapeutic Anti-HER2 Antibody Expression in Vivo," Mol. Ther. 27, no. 8 (Aug. 7, 2019): 1415–23, https://doi.org/10.1016/j.ymthe.2019.05.012.

6. Asha Kumari Patel et al., "Inhaled Nanoformulated mRNA Polyplexes for Protein Production in Lung Epithelium," Adv. Mater. 31, no. 8 (Feb. 22, 2019): 1805116, https://doi.org/10.1002/adma.201970053.

CHAPTER 3

Addressing challenges across the virus like particle manufacturing workflow

The ability to quickly build up vaccine manufacturing for new infectious disease threats has proved valuable during the COVID-19 pandemic, caused by the SARS-CoV-2 virus. Rapid, large-scale vaccine manufacturing matters for existing disease threats, too: pathogens' constant adaptations mean that production of existing vaccines must be agile enough to accommodate mutations.

"Vaccination is one of the most powerful techniques to protect humans and animals from infectious diseases," says Maya Yovcheva, a research scientist at Thermo Fisher Scientific. The World Health Organization estimates that vaccines saved 2-3 million lives in 2019 alone.[1]

Virus like particles are an increasingly popular platform for vaccine manufacturing because of their rapid production and the strength of the resulting immune response. Vaccines based on virus like particles for hepatitis B and human papillomavirus (HPV), which can cause cervical cancer, are available commercially.

Virus like particles are made from viral proteins that self-assemble into a structure resembling a virus's outer shell. These particles do not contain the pathogen's genetic material, so they cannot replicate. Proteins from different strains of the same virus can be included in a virus like particle to strengthen the resulting immune response.

Once a virus has been genetically sequenced, scientists can prepare vaccine candidates using virus like particles more quickly than when using traditional vaccine platforms. One of the most common approaches for making virus like particles is a protein expression system using baculoviruses.

The speed of the baculovirus expression system makes it a promising manufacturing platform to produce vaccines for viruses that mutate rapidly. Scientists at Thermo Fisher Scientific are developing technologies for scalable and cost-effective production, screening, and purification of virus like particles.

Baculovirus-based expression systems

The Gibco ExpiSf™ Baculovirus Expression System is the first baculovirus-based insect system for protein production with components whose ingredients are all known, or chemically defined. It can generate three times as much protein as other insect expression systems.[2]

This protein production kit contains a baculovirus generation kit, insect cells adapted for high-density suspension growth, chemically characterized growth media, and additives to enhance protein expression. All components of the system are engineered to work together for consistent, optimized performance (figure 1).

Figure 1: Components of the Gibco ExpiSf Expression System have been designed to complement one another for rapid, scalable, high yields of protein produced in insect cells via a baculovirus expression system. *Source: Thermo Fisher Scientific*

Scaling protein expression is notoriously challenging, requiring extensive process development to identify conditions that deliver optimal protein yield as cultures get larger.[3] With cells derived to grow well in suspension, this system can be cultured on scales ranging from deep-well plates to medium-volume shake flasks and larger bioreactor vessels. When scaled up from a 25 mL to 400 mL culture volume, the kit produced protein levels largely within expected error, according to data presented in April 2021.[2] Working up to a 10 L culture volume in a 22 L wave bioreactor, cell growth reached

Scaling protein expression is notoriously challenging, requiring extensive process development to identify conditions that deliver optimal protein yield as cultures get larger. — Chantelle Gaskin, Thermo Fisher Scientific

approximately 70% of the growth achieved with shake flasks, which indicates the feasibility of scaling up to a large-batch manufacturing environment.[2]

The media component of modern protein expression systems contains many ingredients that provide nutrients for cell growth and function. This media traditionally contained animal-based serum, but natural variation in those components introduced variation in media performance during biopharmaceutical manufacturing. The biotechnology industry is increasingly developing chemically defined media to provide cell culture conditions that contribute to consistency in protein expression.[4]

The defined ingredients of the Gibco ExpiSf CD Medium provide lot-to-lot consistency, as well as consistent protein expression between cell culture batches. The medium can be used for multiple steps during expression, including cell growth, baculovirus generation, and virus like particle production. It is free from animal components, serum, and protein, which removes concerns about possible negative immune reactions in clinical applications.[5] For large-scale production, the medium is available in a dry powder format created with Thermo Fisher Scientific's granulation technology. According to Yovcheva, the powdered format is an excellent option for a manufacturing environment, as it can be easily dissolved in various volumes of liquid.

Two additives in the Gibco ExpiSf Expression System are also key to consistency and high protein yields. A cationic lipid–based transfection reagent enables high-efficiency gene transfer and corresponding production of high titer baculovirus stocks. The transfection reagent reduces "the need for time-consuming amplification steps and is highly scalable," Yovcheva says. The other additive is a proprietary expression booster, included to maximize protein yields.

The Gibco ExpiSf Expression System offers a greater virus like particle yield from its cells than from conventional cells for both the Chikungunya virus and HPV, according to data in an April 2021 presentation.[2]

Affinity-based capture to speed purification

After retrieving virus like particles from a cell culture, the next step is purification. Conventional workflows often involve multiple purification steps including a number of polishing steps to remove remaining impurities. A major drawback of having several purification steps is loss of product for each of these separation steps, leading to a lower overall yield of the drug product.

A proven approach to minimize product loss is to start out with an affinity-based capture step, says Chantelle Gaskin, field application scientist at Thermo Fisher Scientific. In this step, the product passes through resin with target-specific affinity ligands attached to the resin beads.

Thermo Fisher has developed a diverse collection of affinity ligands, all tuned to bind their specific target molecule with high selectivity and specificity. However, purification of virus like particles for vaccines isn't amenable to off-the-shelf options. While it is possible to develop custom affinity resins to capture vaccine components, such an approach can be time-consuming. One way to make affinity capture generally practical for vaccine manufacturing is to append the target with a molecular tag.

For virus like particles, protein components can be expressed to carry a C-terminal tetrapeptide tag, also called a C-tag. The affinity tag is small enough to minimize potential impact on protein folding and function. In addition, regulatory agencies have approved leaving the tag on the final drug product, streamlining manufacturing.[6] C-tagged products can be easily separated from other components using a specialized chromatography resin that binds to the C-tag, such as Thermo Fisher's CaptureSelect™ C-tagXL resin.

It's worth noting that tag-based capture is shown to be useful in early stages of vaccine development, which typically involves screening of multiple candidates in parallel. A collection of vaccine targets such as various virus like particles, all carrying a C-tag, can be rapidly purified for further testing. Introducing C-tag during drug development streamlines purification strategies overall for successful candidates.

A proven approach to minimize product loss is to start out with an affinity-based capture step

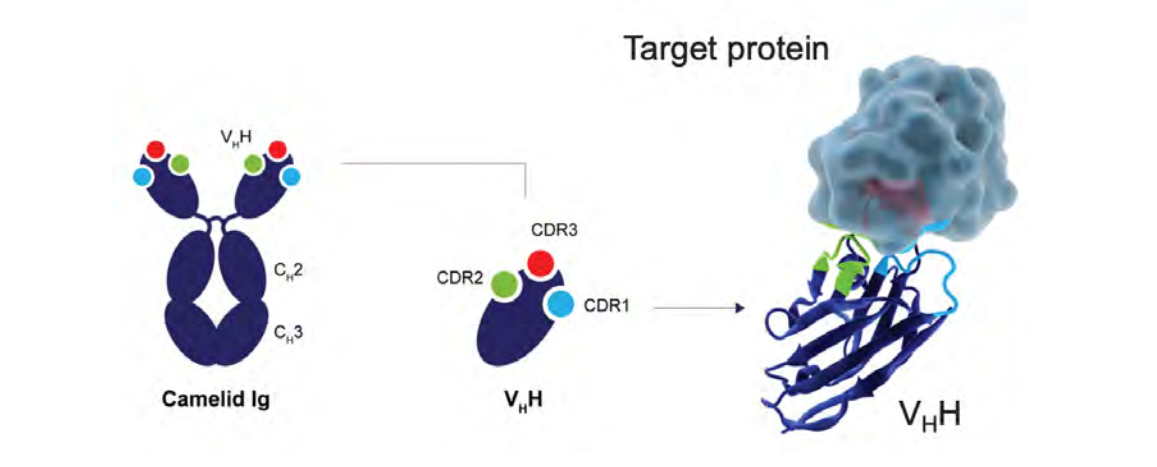
— Chantelle Gaskin, Thermo Fisher Scientific

Using affinity purification for effective polishing

Using the baculovirus-expression system to produce virus like particles poses a unique purification challenge, as the baculovirus and virus like particles are similar in size and envelope structure. These shared traits make baculovirus and virus like particles difficult to separate using traditional purification schemes.

A SMALL LIGAND WITH BIG BENEFITS

Many biotherapeutics lack established affinity purification options. Tunable affinity ligands, such as those used with Thermo Fisher Scientific's CaptureSelect™ affinity resin technology, open the door to new purification possibilities for complex biotherapeutics.



Affinity ligands derived from camelid immunoglobin (Ig) antibodies that have been pared down to the N-terminal domain (V_HH fragment), including just the variable antigen-binding regions, also called complementarity determining regions (CDR), (shown in green, blue, and red). The antigen-binding region of the affinity ligand can be tuned to specifically recognize and capture target molecules, such as proteins. Source: *Thermo Fisher Scientific*

CaptureSelect™ ligands are structurally derived from heavy-chain-only antibodies found in camelids. The variable domain of these antibodies is called the V_HH ligand, and although being small (15 kD), these fragments harbor full antigen-binding capacity. In addition, these ligands are easily tunable through genetic engineering, enabling development of a specific ligand to target virtually any protein, antibody, or viral vector.

V_HH fragments' small size, rigid structure, and tunable recognition makes them useful as affinity ligands in applications that aren't possible with conventional antibodies.

To rapidly and efficiently remove baculovirus impurities from virus like particles produced using baculovirus expression systems, Thermo Fisher has developed the POROS™ CaptureSelect™ BacuClear affinity resin. They started with the POROS™ bead, which has large through-pores that accommodate the purification of larger molecules such as viruses. They added an affinity ligand developed using their CaptureSelect™ technology (See box: A small ligand with big benefits) that specifically captures baculovirus particles

and fragments, leaving virus like particles free to pass through the resin. When researchers purified influenza virus like particles from baculovirus impurities using the BacuClear affinity resin, they found that more than 70 percent of baculovirus was removed from the product.[7]

Quality control testing

All biopharmaceuticals produced in host cells through biotechnology, such as the Sf9/baculovirus expression system, must meet quality control standards set by the World Health Organization (WHO), the US Food and Drug Administration (FDA), the European Union, and other governing regulatory agencies. One of those standards includes a limit on the amount of genetic material from host cells that may be present in a final therapeutic dose.

"Host cell DNA can impact product quality, efficiency, and safety," says Florian Durst, Thermo Scientific's senior field application scientist in the pharma analytics business unit.

Figure 2: ResDNASEQ system integrates rapid sample preparation for nucleic acid extraction with a workflow for real-time quantitative polymerase chain reaction (PCR) analysis. Nucleic acid quantification can be used for rapid impurities and contaminant testing during regulatory quality control in biopharmaceutical production. *Source: Thermo Fisher Scientific*

The WHO and FDA both recommend that residual host cell DNA should be limited to under 10 ng per therapeutic dose.[8,9] The FDA recommends analytical methods for detecting residual DNA have a maximum sensitivity of 10 pg.

In biopharmaceutical manufacturing, residual host cell DNA may be quantified at any step after cell harvest. Testing at multiple stages within a workflow makes the development of a reliable quantification method difficult as it has to be compatible with various sample matrices.

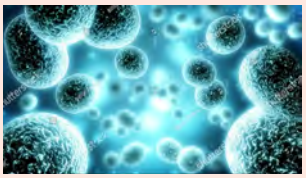
Thermo Fisher has developed kits to quantify DNA from a range of host cells, plasmid vectors, and viruses, including the Sf9 insect cells and baculovirus used in virus like particle production, during biopharmaceutical manufacturing. The company's resDNASEQ™ system provides consistent assay performance via quantitative polymerase chain reaction (qPCR) analysis (figure 2).

For the recently developed duplex Sf9 and baculovirus kit, the resDNASEQ™ workflow has a simultaneous limit of detection of 30 fg and limit of quantification of 300 fg for both baculovirus and SF9 residual DNA. Moreover, the method offers consistent results, as indicated by a coefficient of variation of less than 10%.[2]

Nucleic acid testing can also be used to detect regulated contaminants, such as mycoplasma, which are bacteria that can infect humans and are small enough to pass through most microbial filters.[10] Regulatory agencies worldwide now accept nucleic acid testing to show that processes used for biopharmaceutical production are free of mycoplasma. Previously, the only acceptable method for lot-release mycoplasma detection was a culture-based test that took 28 days.

Thermo Fisher Scientific's MycoSEQ™ Mycoplasma Detection System has been specifically developed and validated to meet regulatory requirements. The real-time, qPCR system can detect more than 90 mycoplasma, spiroplasma, and acholeplasma species without cross-reaction from closely related bacteria, with sensitivity to detect less than 10 copies of mycoplasma DNA per PCR reaction.[2] It has been implemented, and following appropriate validation, accepted by regulatory agencies for more than 40 commercial therapeutics for lot-release testing.

Virus like particles are a powerful platform to generate highly immunogenic vaccines, particularly for viruses that are mutating frequently. An integrated system of tools for protein expression, purification, and quality control testing can help support efficient and cost-effective virus like particle production on a commercial scale.



FOR MORE INFORMATION, watch the webinar "Virus like particle production in insect cells using the baculovirus expression system: Addressing challenges across the workflow" here.

REFERENCES

1. World Health Organization, Vaccines and Immunization, 2019, https://www.who.int/health-topics/vaccines-and-immunization#tab=tab_1.

2. Maya Yovcheva, Chantelle Gaskin, and Florian Durst, "Virus-Like Particle Production in Insect Cells Using the Baculovirus Expression System: Addressing Challenges Across the Workflow," April 15, 2021, webinar, BioProcess International, https://bpi.bioprocessintl.com/virus-like-particle-production-insect-cells-using-baculovirus-expression-system-webcast.

3. Nagesh Tripathi and Ambuj Shrivastava, "Recent Developments in Bioprocessing of Recombinant Proteins: Expression Hosts and Process Development," Front. Bioeng. Biotechnol. 7 (Dec. 20, 2019): 420, https://doi.org/10.3389/fbioe.2019.00420.

4. Nicola McGillicuddy et al., "Examining the Sources of Variability in Cell Culture Media Used for Biopharmaceutical Production," Biotechnol. Lett. 40, no. 1 (Jan. 2018): 5–21, https://www.researchgate.net/profile/Patrick-Floris/publication/319994386_Examining_the_sources_of_variability_in_cell_culture_media_used_for_biopharmaceutical_production/links/59c921e8458515548f3dadda/Examining-the-sources-of-variability-in-cell-culture-media-used-for-biopharmaceutical-production.pdf.

5. Erik Doevendans and Huub Schellekens, "Immunogenicity of Innovative and Biosimilar Monoclonal Antibodies," Antibodies 8, no. 1 (March 5, 2019): 21, https://www.mdpi.com/2073-4468/8/1/21.

6. Rebecca Ashfield and Pim Hermans, "Rapid vaccine manufacturing for clinical trials – Insights from the malaria field," December 9, 2021, webinar, Chemical & Engineering News. https://connect.acspubs.org/CENWebinar_Thermo_12_9_21

7. Mafalda Moleirinho et al., "Baculovirus Affinity Removal in Viral-Based Bioprocesses," Sep. Purif. Technol. 241 (June 15, 2021):116693, https://doi.org/10.1016/j.seppur.2020.116693.

8. World Health Organization, Guidelines on the Quality, Safety, and Efficacy of Biotherapeutic Protein Products Prepared by Recombinant DNA Technology, 2013.

9. US Food and Drug Administration, Center for Biologics Evaluation and Research, Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs)—Guidance for Industry, January 2020, https://www.fda.gov/media/113760/download.

10. M. Hannah Degeling et al., "Sensitive Assay for Mycoplasma Detection in Mammalian Cell Culture," Anal. Chem. 84, no. 9 (May 1, 2012): 4227–32. https://pubs.acs.org/doi/pdf/10.1021/ac2033112.

CHAPTER 4

Affinity tags head toward the vaccination clinic

Protein purification is a tricky business. Separating a desired protein from a soup of other proteins—such as in the media used to grow cells—is particularly taxing. Generic column-based purification procedures, which exploit differences in protein size and charge, tend to give imperfect results. “You often have to use several different steps to get sufficient protein purity,” says Rebecca Ashfield, senior project manager at the Jenner Institute.

Affinity chromatography was developed as an alternative, a more targeted purification approach than these generic procedures. It has been a laboratory staple for decades. The affinity resins contain components, such as antibodies or heavy metal ions, which bind to desired proteins. When a crude mixture is passed through an affinity resin, the desired protein is trapped. Other proteins and impurities flow through the column before the target protein is released and collected.

The technique was initially limited to purifying protein types for which a suitable resin had been developed. Around 30 years ago, affinity tags were introduced to significantly expand the types of proteins compatible with this purification strategy. This approach involves attaching a short peptide sequence—known as the affinity tag—to a target protein using genetic engineering. The resin recognizes the affinity tag. It is a useful one-step technique that results in a pure protein, Ashfield says.

Typically, however, researchers avoid using affinity tags in the purification of therapeutic proteins. First, cleaving off the affinity tag during manufacture is not generally an option in line with good manufacturing practice (GMP) regulations that are in place to ensure the safety, purity, and effectiveness of pharmaceuticals. And leaving extra material on a protein destined for use as a vaccine is usually frowned upon because of the possibility of antibodies being made in the body against the affinity tag rather than the therapeutic protein, Ashfield explains.

She and her colleagues recently gained approval to leave one very short affinity tag—just four amino acids long—on a malaria vaccine candidate for clinical trials. This has opened the door to a range of novel protein-based malaria vaccine candidates that were proving impossible to purify in any other way. These agents are all either in or progressing toward clinical trials. The team is working with the assumption that the tag will be able to remain

in any vaccines that are eventually licensed. The molecules could someday soon provide protection against malaria, a mosquito-borne disease that still kills about 435,000 people globally each year.

Progress on a malaria vaccine

Malaria cases have dropped significantly since 2010, thanks to insecticide-treated mosquito nets, indoor residual spraying of insecticides, and antimalarial drugs. But progress has plateaued in recent years. There are still over 200 million clinical cases of malaria every year worldwide. “We desperately need a vaccine to add to the established control measures,” says Simon Draper, professor of vaccinology and translational medicine and Ashfield’s colleague at the Jenner Institute.

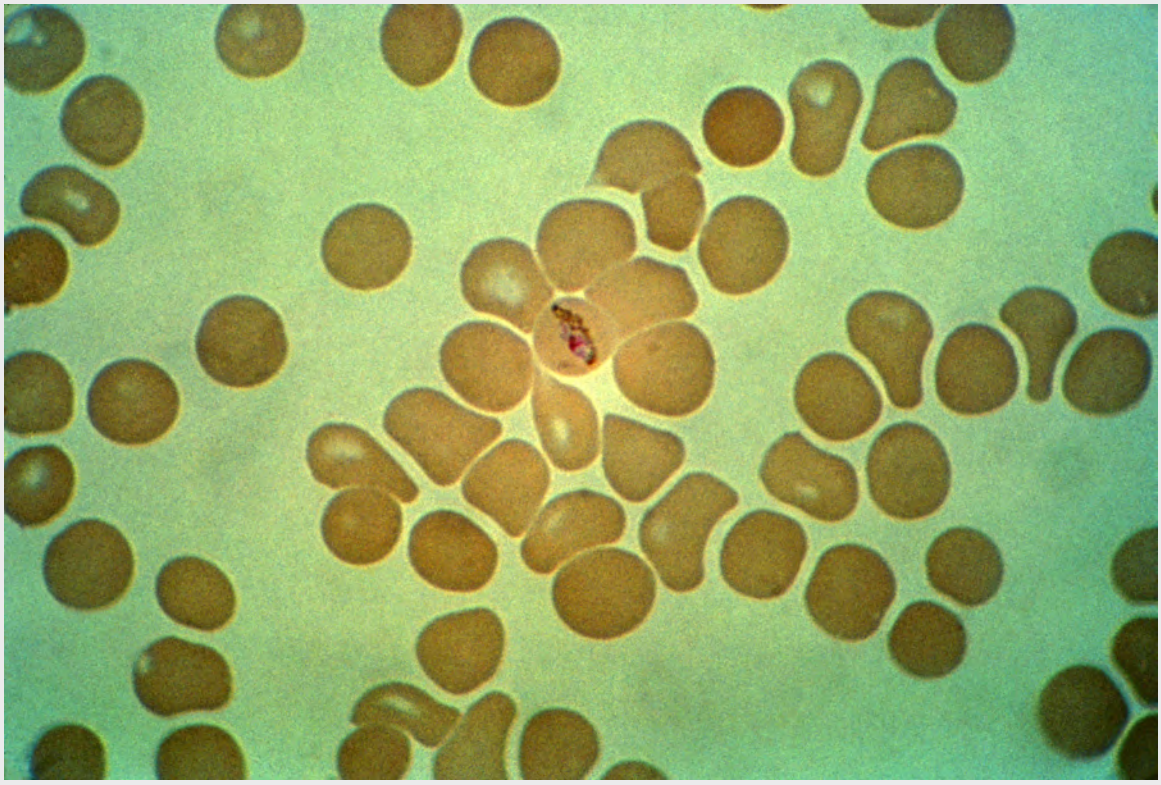
No malaria vaccines have been approved, but many are in development. The most advanced is GlaxoSmithKline’s RTS,S/AS01, which targets the pre-erythrocytic stage of the malaria parasite’s life cycle (when it enters and replicates in the affected person’s liver). The vaccine candidate is a virus-like particle (VLP), which means it is a collection of proteins forming a particle that resembles a virus and can trigger an immune response without the risk of infection. Clinical trials showed that the particle has modest efficacy. It is being introduced in selected areas of Ghana, Kenya, and Malawi as part of a large pilot implementation program. Vaccines with greater efficacy continue to be pursued.

Another promising vaccine candidate is RH5.1.[1,2] It targets the blood-stage of the life cycle, when the parasite has left the liver and entered the blood. “It’s difficult to stop the parasite invading red blood cells because it has lots of backup strategies to get into the cell,” Draper explains. “So if you hit it one way, it has a backup and then another backup.” In 2010, he adds, “it was discovered that there was one protein, called RH5, which the parasite absolutely needs to get into the red blood cells, and there’s no backup.”

RH5 is secreted by the parasite and serves as a connector, bonding with a protein on the surface of a red blood cell. RH5 thus forms a physical link between parasite and erythrocyte, giving the invader the opportunity to slip inside the red blood cell. Antibodies that block RH5 from connecting with these surface proteins disrupt the invasion process. “The idea of the vaccine is that you introduce antibodies against RH5 in the person that you vaccinated,” Draper says. “Thereby if they ever get infected with malaria for real, it can’t infect their red blood cells and therefore can’t make them sick.”

RH5 proved difficult to make in the laboratory, however. “It took us a long time to crack that problem and discover that you can make the RH5 protein in insect cells and express

it into the supernatant," Draper says. "The problem with insect cells is that the supernatant that you grow the cells in is very rich. So we had difficulty purifying it using traditional technologies."



Light micrograph showing the parasitic protozoa Plasmodium malariae (see the most central blood cell) which causes malaria in humans; Magnification 1000x at 35mm. *Image credit: Science Source*

At this point, the researchers approached Thermo Fisher Scientific for help. "We teamed up with them because they had this new purification system called the C-tag," Draper says. In collaboration with researchers from the VIB in Belgium, Jan Steyaert and Els Pardon, the company developed an affinity resin (CaptureSelect™ C-tag Affinity Matrix) that included an antibody that could capture any protein with a specific four-residue sequence on its C-terminus—the C-tag (See box: Discovering C-tag).

The Jenner Institute scientists put a C-tag sequence on the end of the RH5 protein and tried purifying it using the CaptureSelect™ C-tag chromatography resin. "We discovered it was a fantastic means by which to pull the RH5 protein out of the rich supernatant

To use an affinity resin for manufacturing of a therapeutic molecule—one that will be injected into patients or healthy volunteers—the quality level of the resin must be higher than for research use only," says Pim Hermans, director of ligand discovery at Thermo Fisher Scientific. — Pim Hermans, Thermo Fisher Scientific

mixture from the insect cells," Draper says. The team achieved a greater than 85% recovery and greater than 70% purity in a single step.[2]

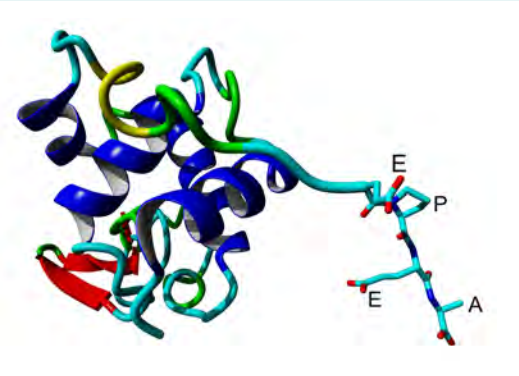
That wasn't the end of the story, however, as the CaptureSelect™ resin was approved only for research use. "To use an affinity resin for manufacturing of a therapeutic molecule—one that will be injected into patients or healthy volunteers—the quality level of the resin must be higher than for research use only," says Pim Hermans, director of ligand discovery at Thermo Fisher Scientific. His team worked to redesign its resin to significantly increase its binding capacity and meet all GMP requirements.[1]

With the regulatory hurdles cleared, RH5.[1] was to be the first protein vaccine with an attached C-tag to enter clinical trials. It has completed safety and small-scale efficacy trials in the UK, and Draper hopes to start a trial in Tanzania next year. "We're confident after having a discussion with the regulatory authorities that it's not going to be an issue throughout clinical development to use this particular tag," Ashfield says.

DISCOVERING C-TAG

Several small affinity tags are currently being used in the laboratory. Some of these, like C-tag and FLAG-tag, utilize antibody-filled chromatography resins. Others, such as polyhistidine tags, work based on the strong interaction of histidine residues with heavy metal ions, such as zinc, coupled to a resin.

The C-tag, a four-amino-acid-long sequence (glutamic acid-proline-glutamic acid-alanine), is the smallest affinity tag that can be fused at the C-terminus of any recombinant protein. A single-domain camelid antibody with high affinity and selectivity for this short peptide sequence was identified around a decade ago[4] by scientists from the VIB in a study headed by Christopher M. Dobson from the University of Cambridge.



The C-tag, a sequence of glutamic acid (E), proline (P), glutamic acid (E), and alanine (A), is the smallest affinity tag that can be fused at the C-terminus of a recombinant protein. Here, it is fused to lysozyme. *Image credit: Thermo Fisher Scientific*

The team was studying how the α-synuclein protein aggregates to form the amyloid structures known to participate in neurological disorders, such as Parkinson's disease. They mixed antibody fragments with α-synuclein and looked for any resulting folding changes. "What they observed was that one of these antibodies was selectively targeting the C-terminal end of the α-synuclein protein," Hermans says. "The crystal structure showed that the four residues at the C-terminal end were largely captured by this antibody fragment, and it was really deep down in the binding pocket."

The scientists wondered if this combination of high selectivity and tight binding meant that the four amino acid sequence at the C-terminus of α-synuclein had the potential to be an antibody-based affinity tag. The VIB approached Hermans's group at BAC (now a part of Thermo Fisher Scientific). "They knew that we are making products for affinity purification," Hermans says. "And with the support of Jan Steyaert and Els Pardon this led to the development of a new type of affinity tag resin."

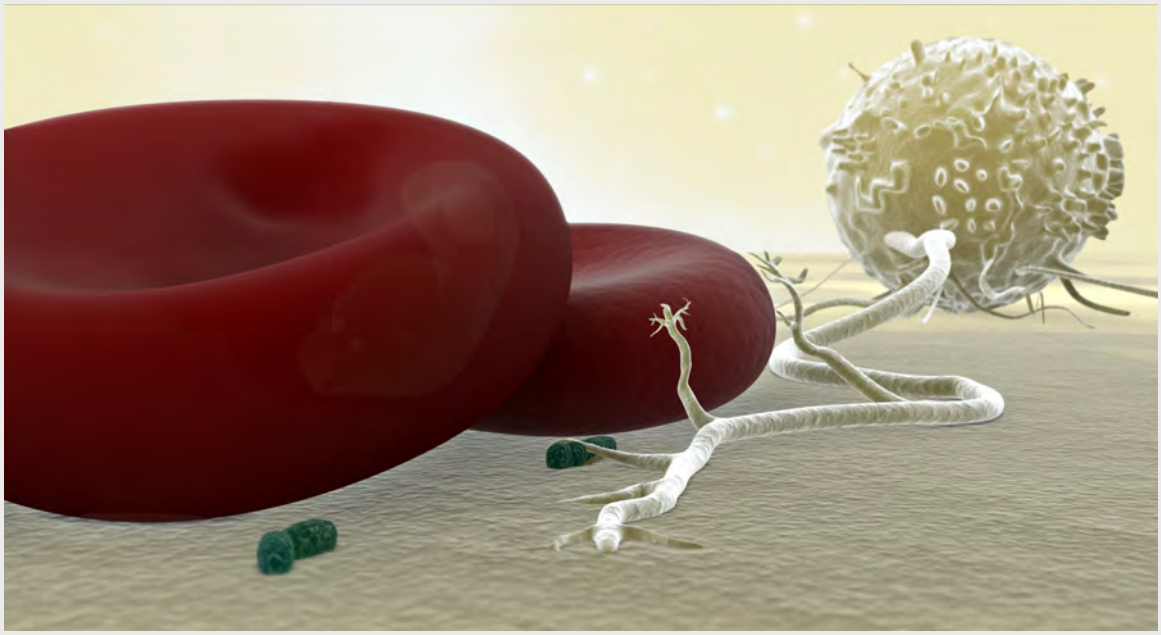
C-tag offers a number of advantages over other affinity tags, he adds. Its small size means that, unlike some of the larger tags, it doesn't alter protein folding and functionality. It has also been shown to punch above its weight in terms of purity achieved. For example, researchers at the Jenner Institute found that the purity of their C-tagged vaccines was far higher than observed when they used a polyhistidine-tag.[2] C-tag purification also has environmental advantages: the C-tag resin doesn't contain metal ions, so there is no heavy metal waste.

We're confident after having a discussion with the regulatory authorities that it's not going to be an issue throughout clinical development to use this particular tag. — Rebecca Ashfield, Jenner Institute

A vaccine technology platform

At the Jenner Institute, the C-tag system is now a go-to approach for purifying protein vaccine candidates to meet GMP standards. It's much quicker and cheaper to develop the manufacturing process for a novel vaccine when researchers know generally how to purify the proteins, according to Draper. "You haven't got to work it out from scratch in a bespoke manner each time," he says.

A second malaria vaccine candidate purified with C-tag, R21c, is in clinical trials. "We were struggling to manufacture this second vaccine in a way that met GMP requirements before we tried using a C-tag," Ashfield says. Developed by Professor Adrian Hill, director of the Jenner Institute, R21c is similar in concept to GSK's RTS,S/AS01: it's a virus-like particle that targets the pre-erythrocytic stage of the malaria parasite life cycle. R21c is in early stage trials in Kenya, the UK, and Burkina Faso.[3]



Computer artwork of the malarial parasite Plasmodium falciparum infecting blood cells.
Image credit: Science Source

Draper's lab is also using the C-tag system for a second-generation RH5.1 malaria vaccine. "There are two other proteins that we now have learned form a complex with RH5, so we are also making both of those targets as well," Draper says. They will be manufactured very much like RH5.1—grown in insect cells with C-tags attached.

"Our ultimate ambition is to stick those proteins onto a virus-like particle with the hope it will give us a stronger immune response," he says.

Though to this point the Jenner Institute has used the C-tag system only for malaria vaccines, Draper foresees it finding much wider clinical use. "This isn't a specific technology for malaria," he says. "If you were making a vaccine for HIV or tuberculosis or Ebola or cancer or whatever, the C-tag could potentially be useful. It could be applied to lots of different vaccines, irrespective of the disease that you're targeting."

REFERENCES

1. J. Jin et al., "Accelerating the Clinical Development of Protein-Based Vaccines for Malaria by Efficient Purification Using a Four Amino Acid C-Terminal 'C-tag.'" Int. J. Parasitol. 47, no. 7 (June 2017): 435–446, https://doi.org/10.1016/j.ijpara.2016.12.001.

2. J. Jin et al., "Production, Quality Control, Stability, and Potency of cGMP-Produced Plasmodium falciparum RH5.1 Protein Vaccine Expressed in Drosophila S2 Cells," npj Vaccines 3, article no. 32 (Aug. 2018), https://doi.org/10.1038/s41541-018-0071-7.

3. K. A. Collins et al., "Enhancing Protective Immunity to Malaria with a Highly Immunogenic Virus-like Particle Vaccine," Sci. Rep. 7, article no. 46621 (April 2017), https://doi.org/10.1038/srep46621.

4. E. J. De Genst et al., "Structure and Properties of a Complex of α-Synuclein and a Single-Domain Camelid Antibody," J. Mol. Biol. 402, no. 2 (Sept. 2010): 326–343, https://doi.org/10.1016/j.jmb.2010.07.001.

CHAPTER 5

How affinity tags are speeding up malaria vaccine development

Source: Thermo Fisher Scientific

In 2020, there were 241 million malaria cases and 627,000 malaria deaths worldwide, according to the World Health Organization (WHO).[1] About 95% of the cases occurred in Africa, with most deaths in this region involving children under 5.

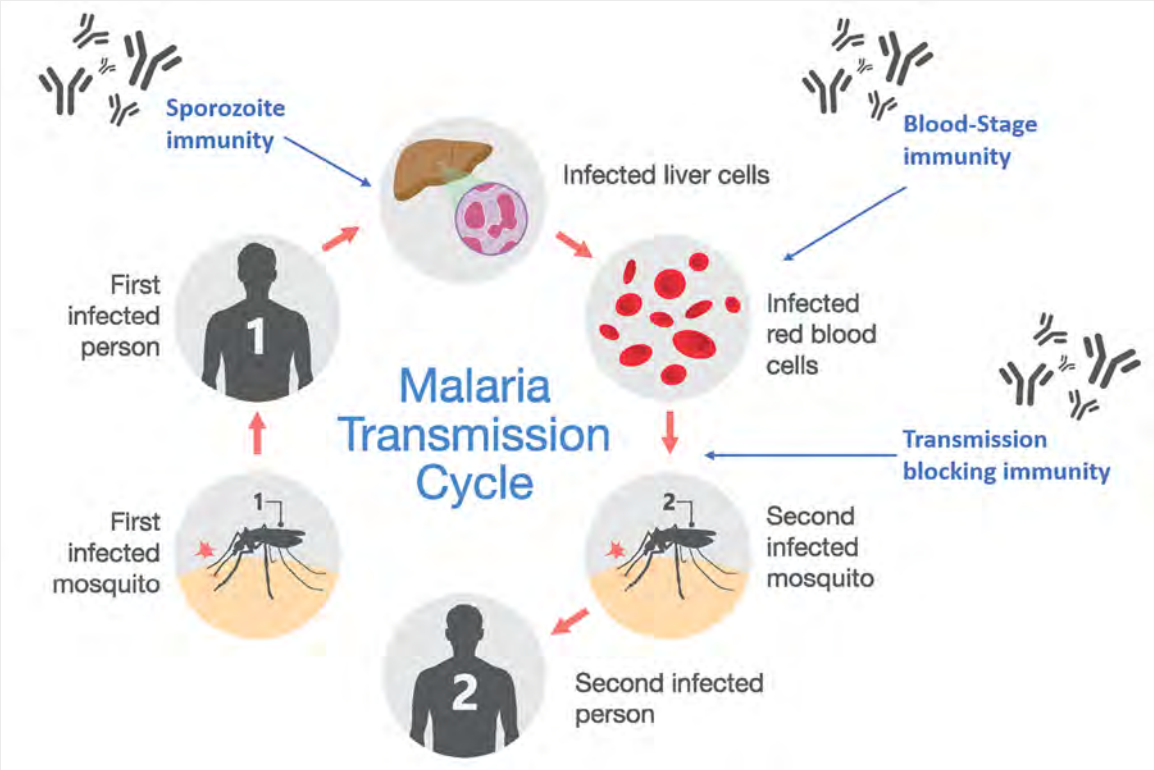
"Most of the deaths were caused by *Plasmodium falciparum*, one of the five malaria parasites that can cause disease in humans," explains Rebecca Ashfield, a senior project manager at the University of Oxford's Jenner Institute.

On October 6, 2021, the WHO approved a malaria vaccine for the first time: RTS,S/AS01. The vaccine is a viruslike particle, a construct that closely resembles a virus but contains no genetic material and therefore isn't infectious. It's known under the tradename Mosquirix but is better known as RTS,S. The WHO recommended the vaccine for widespread use in children living in sub-Saharan Africa and other regions with moderate to high *P. falciparum* malaria transmission rates. This approval was the cumulation of over 30 years of research and development by GlaxoSmithKline, the nonprofit health organization Path, and other partners, including the Bill and Melinda Gates Foundation. "In field trials in Africa, RTS,S is approximately 30–50% effective," Ashfield says. "It's a very good start."

But researchers have no intention at stopping there, and Ashfield and her colleagues have a pipeline of malaria vaccines in development that they hope will boast even higher efficacy. The manufacture of recent Oxford malaria vaccine candidates follow a blueprint that includes a novel purification approach for a biotherapeutic: chromatography using an affinity tag system. The affinity tag has enabled the team to overcome issues with purity, keep costs down, and rapidly advance its vaccine candidates into the clinic.

Testing times

Ten COVID-19 vaccines have been approved by the WHO since the coronavirus that causes it was discovered in humans just a few years ago, while scientists have spent decades working toward effective malaria vaccines. What's holding things up in the malaria field?



The vaccines being developed for malaria are each designed to disrupt one of three different stages of the *P. falciparum's* complicated life cycle: how it infects liver cells, how it multiplies within red blood cells, and how it transmits to a new host. *Source: Thermo Fisher Scientific*

The overall strategy is to test as many different candidates as possible in the clinic, because at the moment, we don't know whether a vaccine is going to be effective until we test it in a clinical trial.

— Rebecca Ashfield, University of Oxford's Jenner Institute

For one, malaria research isn't particularly well funded.[1] But even for those with the grants, a lack of a clear biological target on *P. falciparum* remains a huge challenge for malaria vaccine developers. "*Plasmodium falciparum* has about 7,000 genes, about 25% of the number of a human," Ashfield says. "So unlike a virus—for example, SARS-CoV-2, where it was obvious that we had to target the spike protein on the surface of the virus—it is by no means obvious which of the parasite's 7,000 genes would make a good subunit vaccine."

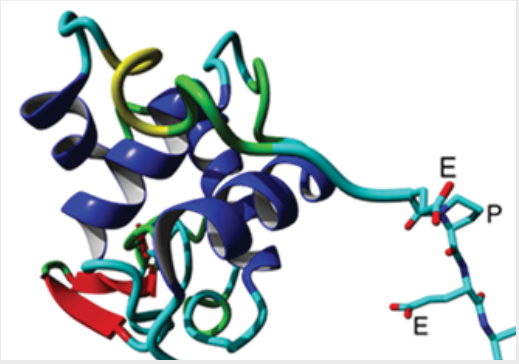
That lack of a biological target hinders researchers' ability to assess predictions of suitable vaccine designs in the lab. The Oxford team has concluded that clinical trials are the only appropriate way of evaluating hypotheses. "The overall strategy is to test as many different candidates as possible in the clinic, because at the moment, we don't know whether a vaccine is going to be effective until we test it in a clinical trial," Ashfield says.

The Oxford team has three vaccine *P. falciparum* candidates in clinical trials, each targeting a different stage of the parasite's life cycle. (The life cycle of this parasite is particularly complex and involves both the mosquito and human hosts.)

Tags to the rescue

Figuring out how to manufacture vaccine candidates in accordance with good manufacturing practices (GMPs) without breaking the bank was a significant challenge for the Oxford team. For an academic group, vaccine manufacturing development can be prohibitively expensive, Ashfield says.

The Oxford scientists devised a standardized manufacturing blueprint while developing the manufacturing route to one of their vaccine candidates—the recombinant protein RH5.1.[2] The goal was to speed up manufacture and increase yields, thereby decreasing short-term costs. That blueprint has since been used, at least for early-stage manufacture, for all three of the vaccines in clinical trials and for a handful of others that are close to reaching the clinic.



The C-tag, a sequence of glutamic acid (E), proline (P), glutamic acid (E), and alanine (A), is the smallest affinity tag that can be fused at the C-terminus of recombinant proteins. Thermo Fisher produces a C-tag affinity resin suitable for GMP manufacture. *Source: Thermo Fisher Scientific*

To make a vaccine, the antigen—the biomolecule that stimulates the immune response in the body—must first be cultivated in a bacteria, yeast, or cell culture in higher eukaryotes. Using a technology developed in collaboration with the Danish firm ExpreS2ion Biotechnologies, the Oxford team grows many of its recombinant proteins (including RH5.1) in *Drosophila* Schneider 2 cells. “This particular cell line is very good at refolding plasmodium proteins that are difficult to express in other systems,” Ashfield says. The others are cultivated in the yeast *Pichia pastoris*.

The next step in vaccine manufacture is to fish the antigen out of the crude growth medium and purify it. The growth-medium soup contains a complex mixture of biomolecules, and selectively pulling out just one of these can be highly challenging. Traditionally, chromatography columns separate mixtures by exploiting differences in their sizes or charges. Differences between biomolecules in these soups can be extremely small, however, so multiple different column types must be used in succession to meet GMP purity requirements. Developing the methodology and executing a purification protocol with multiple steps is time consuming, and each additional step reduces the yield of the desired antigen, according to Pim Hermans, head of ligand discovery at Thermo Fisher Scientific.

Affinity chromatography is an established tool for reducing the number of steps required when biomolecules are being purified. Rather than relying on size or charge differences, affinity columns separate biomolecules according to how well they bind—their affinity—to heavy metal ions or to other biomolecules immobilized on the chromatography resin. As the mixture passes through the column, biomolecules with high affinity for the immobilized molecules bind to the resin. After everything else has passed through the column, the eluting solvent is changed to release the captured biomolecules. For GMP manufacturing, one or two polishing chromatography rounds to remove final traces of impurities will likely be all that’s needed.

INTRODUCING C-TAG

The C-tag is a short peptide sequence of four amino acids (E-P-E-A) that can be attached to the carboxy-terminus (the free carboxyl group, better known as the C-terminus) of any recombinant protein. The potential to use this tag in affinity chromatography was identified over a decade ago by the University of Cambridge chemist Christopher M. Dobson and his collaborators.[6]

The immobilized biomolecules on the chromatography resin that capture C-tags are based on a pared-down version of single-domain immunoglobulin G (IgG) fragments.

Using a recombinant fragment rather than the whole antibody has multiple advantages. Fragments of single-domain antibodies are more robust and stable than whole ones, Hermans says. “They can also be expressed in microbial systems, like the yeast system that we use, in high yields,” he adds.

Thermo Fisher started manufacturing its CaptureSelect C-tag affinity resin in 2012, at first for research purposes only. It wasn’t until the collaboration with Ashfield and the other malaria vaccine developers at Oxford that Hermans and his team seriously considered the possibility of their C-tag system being used for biotherapeutic manufacture. “We worked together to make this resin suitable for large-scale purification,” Hermans says.

The hope is that the success of the malaria vaccines’ GMP manufacture using a C-tag will open the door for other vaccine developers, targeting a wide range of diseases, to do the same and get their much-needed vaccines to populations at risk of infection faster.

The most well-known affinity resin contains immobilized protein A, a bacterial surface protein with high affinity toward immunoglobulins. Protein A affinity resins are widely used in the manufacture of therapeutic monoclonal antibodies. Affinity chromatography’s potential has been limited by an inherent problem: challenges associated with making affinity resins for other types of biologics. “For the majority of biomolecules, no affinity capture resin is available,” Hermans says.

About 30 years ago, a workaround was devised: affinity tags. A short peptide sequence—the affinity tag—is attached to the target biomolecule using genetic engineering. The target can then be captured by an affinity tag–specific ligand that’s attached to the resin. Once the target biomolecule has been released from the affinity column, the tag can be removed.

Affinity tags are now used extensively in the research lab. So far, however, safety concerns have hampered their use in biotherapeutic manufacture. Removing affinity tags during a biotherapeutic manufacturing process is laborious and can lead to product loss. In addition, if the tag is left on a biotherapeutic, it's possible that antibodies may be made in the body against the affinity tag rather than the therapeutic molecule.

While struggling to develop a traditional chromatography GMP process for RH5.1, the Oxford team looked at the possibility of utilizing an affinity tag. The scientists tested some commercial affinity tag systems in the research lab, and identified one—the C-tag—that met their purity requirements.

The C-tag is four amino acids long—glutamic acid (E), proline (P), glutamic acid (E), alanine (A)—and believed to be the smallest tag for which an affinity resin has been created. Thermo Fisher developed the affinity chromatography resin for the capture of C-tagged biomolecules under the brand name CaptureSelect™ C-tagXL Affinity Matrix.

The purity achieved using the C-tag system is very high, and the resin "is really exquisitely specific," Ashfield says. "It really does not bind to the other proteins." All that's needed to meet GMP purity requirements for the Oxford team's vaccines manufactured in *Drosophila* Schneider 2 cells is one polishing step using size-exclusion chromatography and a virus filtration step (to remove viruses in the growth medium). The yields are also pleasing, Ashfield says. For RH5.1, the C-tag chromatography step has an 85% yield.

Another reason the scientists selected the C-tag system was because they predicted that the four-amino-acid-long tag was too small to elicit unwanted immune response in the body. They were therefore hopeful that the regulatory authorities would allow the tags to remain permanently on their vaccines, which eliminates the need for a cleaving step. "We consulted with the MHRA [Medicines and Healthcare products Regulatory Agency], the regulatory body in the UK, before we took RH5.1 into a clinical trial, and they indicated that they were very happy for the C-tag to be used throughout

It's such a small sequence—and indeed it's smaller than an antibody epitope—so it's hard to see how you'd get a lot of immunogenicity building up against that tag. — Rebecca Ashfield, University of Oxford's Jenner Institute

development," Ashfield says. "It's such a small sequence—and indeed it's smaller than an antibody epitope—so it's hard to see how you'd get a lot of immunogenicity building up against that tag."

Once it looked likely that leaving the C-tag on for clinical trials would be approved, Hermans's team at Thermo Fisher redesigned the CaptureSelect™ resin to boost its binding capacity and meet other GMP requirements.[2]

Vaccines in the field

The approved GlaxoSmithKline RTS,S malaria vaccine intervenes immediately after a mosquito bites a human and releases sporozoite parasites into the bloodstream. The vaccine's goal is to prevent the parasite from reaching and infecting the liver cells, where it multiplies and reenters the bloodstream to infect red blood cells and cause disease symptoms.

The Oxford team has also developed a vaccine, R21, targeting that same stage in *P. falciparum's* life cycle. This vaccine's development is spearheaded by Ashfield's colleague Adrian Hill. R21 is similar to RTS,S in many ways and has shown over 70% efficacy in Phase 2b field trials in Burkina Faso.[3] Further clinical trials are underway there, as well as in Kenya, Mali, and Tanzania.

While C-tag was initially used for the GMP manufacture of R21, it isn't currently employed for that purpose. "For R21, we moved away from C-tag purification because large-scale manufacture was transferred to the Serum Institute of India and they preferred a process that didn't involve affinity purification," Ashfield says. The current C-tag resins work better with small soluble proteins than larger virus-like-particles. "Thermo Fisher is developing different purification resins to cope with this issue," she adds.

The next stage of the *P. falciparum* life cycle that the Oxford vaccine makers are targeting is multiplication within red blood cells. The *P. falciparum* multiplies inside the cells until they burst and release the parasites, which go on to invade other red blood cells.

Simon Draper is leading the Oxford effort to develop vaccines that hinder parasite multiplication in red blood cells. The first-generation vaccine candidate of this type is RH5.1. The standardized capture-and-purification GMP process involving C-tag was designed during RH5.1's development.

Table 1: Malaria vaccine overview

Target in parasite life cycle	Vaccine name	Lead developer	Vaccine type	Development progress
Sporozoite stage	RTS,S/AS01	GlaxoSmithKline	Viruslike particle	Approved by WHO in October 2021
Sporozoite stage	R21	Adrian Hill (Oxford)	Viruslike particle	Phase 3
Blood stage	RH5.1	Simon Draper (Oxford)	Soluble protein	Phase 1b
Blood stage	RH5.2	Simon Draper (Oxford)	Viruslike particle	Scheduled to start trials
Blood stage	CyRPA-Ripr	Simon Draper (Oxford)	Fusion protein	Scheduled to start trials shortly
Transmission stage	Pfs25-IMX313	Sumi Biswas (Oxford)	Nanoparticle	Phase 1b
Transmission stage	Pfs48/45	Sumi Biswas (Oxford)	Soluble protein	Scheduled to start trials shortly

A Phase 1/2a UK study of the vaccine in healthy adults demonstrated that it was safe and well tolerated and that antibodies against malaria remained in the body for over 2 years after treatment.[4] A Phase 1b clinical trial for RH5.1 began in early 2021 in Tanzania.

Two other candidates that target parasite multiplication in red blood cells, the virus like particle RH5.2 and the fusion protein CyRPA-Ripr, are expected to start trials shortly.

The Oxford team is also trying to inhibit the transmission stage of the *P. falciparum* life cycle, when the mosquito collects parasite-laden blood from a person with malaria and transfers it to a new host. Stopping transmission means that though the individual who was originally vaccinated isn't protected from disease, "other individuals in the community will be protected when they're bitten by the mosquito," Ashfield says. "This is a way of building up herd immunity."

Sumi Biswas leads the Oxford transmission-blocking malaria vaccine project. A UK-based Phase 1a clinical trial of the first vaccine candidate of this type—the nanoparticle Pfs25-IMX313—recently finished.[5] A Phase 1b trial began in Tanzania in mid-2021.

The GMP manufacturing process using C-tag has also been completed for a second transmission-blocking vaccine candidate, the soluble protein Pfs48/45. The first clinical trial is scheduled for this year, according to Ashfield.

The Oxford team has other *P. falciparum* vaccine candidates in earlier stages of development. Ashfield predicts that eventually people will receive multiple malaria vaccines targeting different stages of the P. falciparum life cycle. The team has secured funding for clinical trials of combinations of its vaccines. "We're very hopeful that that's going to be the way forward," Ashfield says. The vaccines initially will be made separately and mixed in the clinic but in time will be prepared in one formulation, similar to the MMR (measles, mumps, and rubella) vaccine.

REFERENCES

1. World Malaria Report 2021, World Health Organization, Dec. 6, 2021, https://www.who.int/publications/i/item/9789240040496.

2. Jing Jin et al., "Accelerating the Clinical Development of Protein-Based Vaccines for Malaria by Efficient Purification Using a Four Amino Acid C-Terminal 'C-Tag,' " Int. J. Parasitol. 47, no. 7 (June 2017): 435–46, https://doi.org/10.1016/j.ijpara.2016.12.001.

3. Mehreen S. Datoo et al., "Efficacy of a Low-Dose Candidate Malaria Vaccine, R21 in Adjuvant Matrix-M, with Seasonal Administration to Children in Burkina Faso: A Randomised Controlled Trial," Lancet 397, no. 10287 (May 15, 2021): 1809–18, https://doi.org/10.1016/S0140-6736(21)00943-0.

4. Angela M. Minassian et al., "Reduced Blood-Stage Malaria Growth and Immune Correlates in Humans Following RH5 Vaccination," Med 2, no. 6 (June 11, 2021): 701–19, https://doi.org/10.1016/j.medj.2021.03.014.

5. Hans de Graaf et al., "Safety and Immunogenicity of ChAd63/MVA Pfs25-IMX313 in a Phase I First-in-Human Trial," Front. Immunol. 12, article no. 694759 (July 2021), https://doi.org/10.3389/fimmu.2021.694759.

6. Erwin J. De Genst et al., "Structure and Properties of a Complex of α-Synuclein and a Single-Domain Camelid Antibody," J. Mol. Biol. 402, no. 2 (Sept. 2010): 326–43, https://doi.org/10.1016/j.jmb.2010.07.001.

7. C. Hamers-Casterman et al., "Naturally Occurring Antibodies Devoid of Light Chains," Nature 363 (June 3, 1993): 446–48, https://www.nature.com/articles/363446a0.