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.”

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

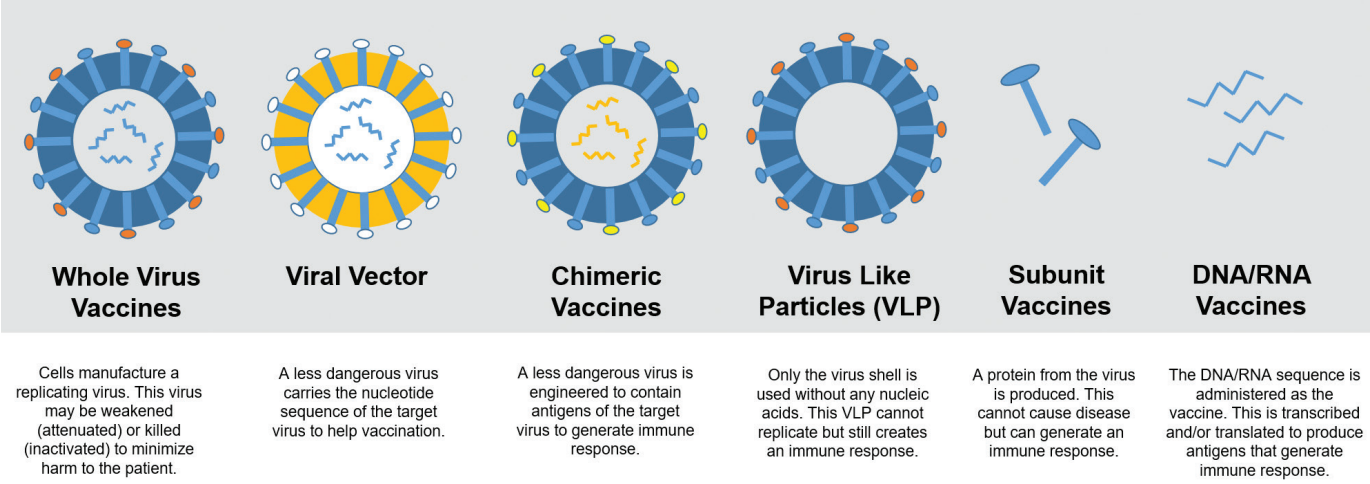

Figure 1: Vaccines vary in composition but include at least one molecular antigen or nucleic acid sequence that encodes an antigen capable of generating a specific immune response. Each vaccine type presents different manufacturing and processing challenges.

Source: Thermo Fisher Scientific

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

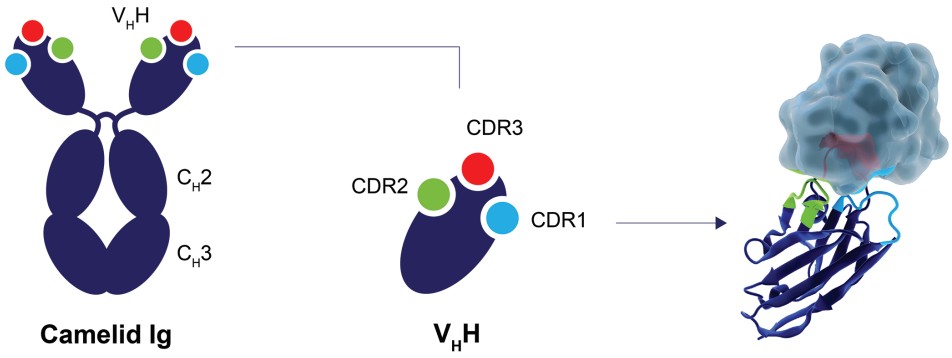

Figure 2: The CaptureSelect affinity ligands are derived from camelid immunoglobulin (Ig) antibodies that have been pared down to the N-terminal domain (VHH fragment), including just the variable antigen-binding regions, also called complementarity determining regions (CDR), (shown in green, blue, and 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

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.

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.

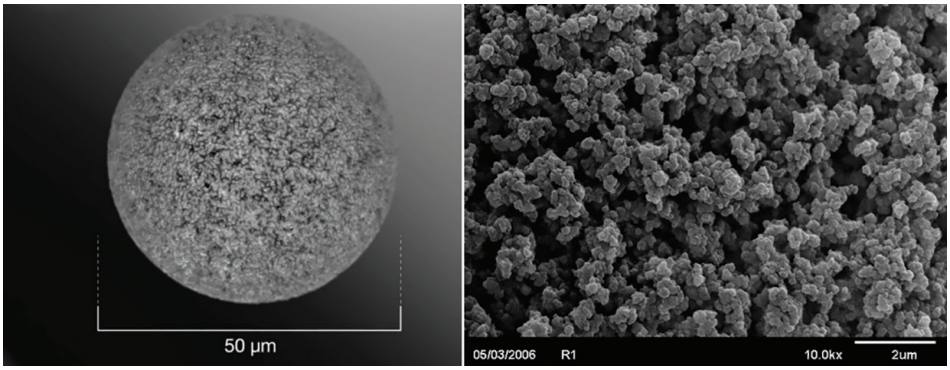

Figure 3: Scanning electron microscope images show a POROS resin bead on the left, with a magnified image on the right showing the large through-pores. These allow for increased surface area and therefore more binding capacity while maintaining the ability to separate out the target molecule from impurities.

Source: Thermo Fisher Scientific

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

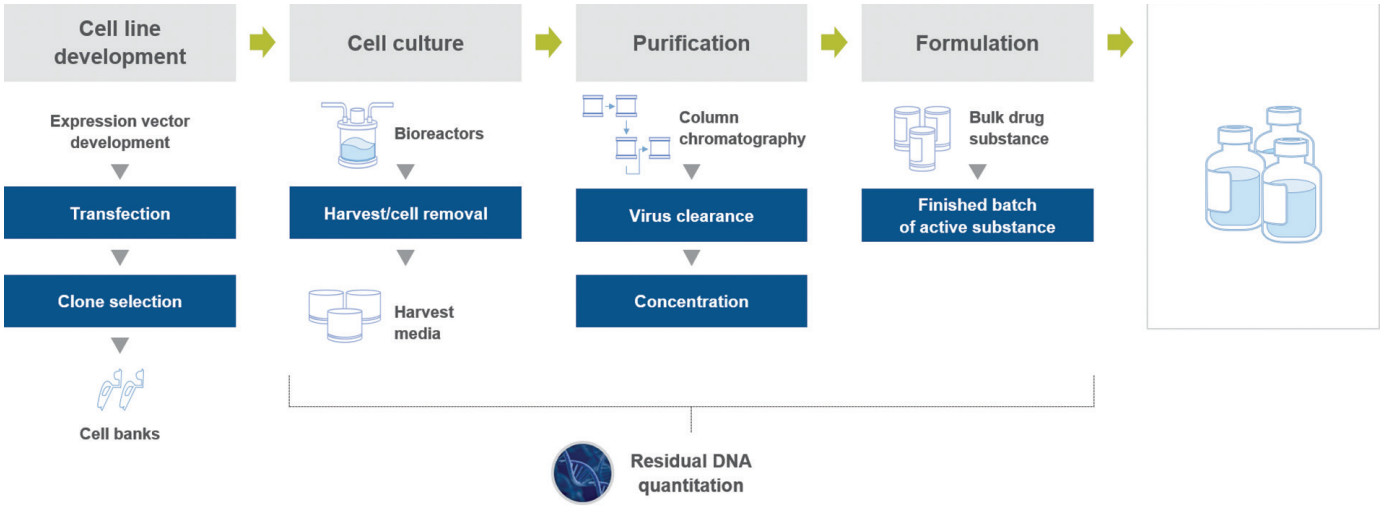

Figure 4: Vaccines need to be tested for residual DNA impurities in the final dosage form, while such testing during manufacturing can help optimize and monitor the process. Quick, reliable assays for residual host DNA can help speed up development of vaccine manufacturing processes, helping finished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

Source: Thermo Fisher Scientific

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."

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 a webinar here.

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