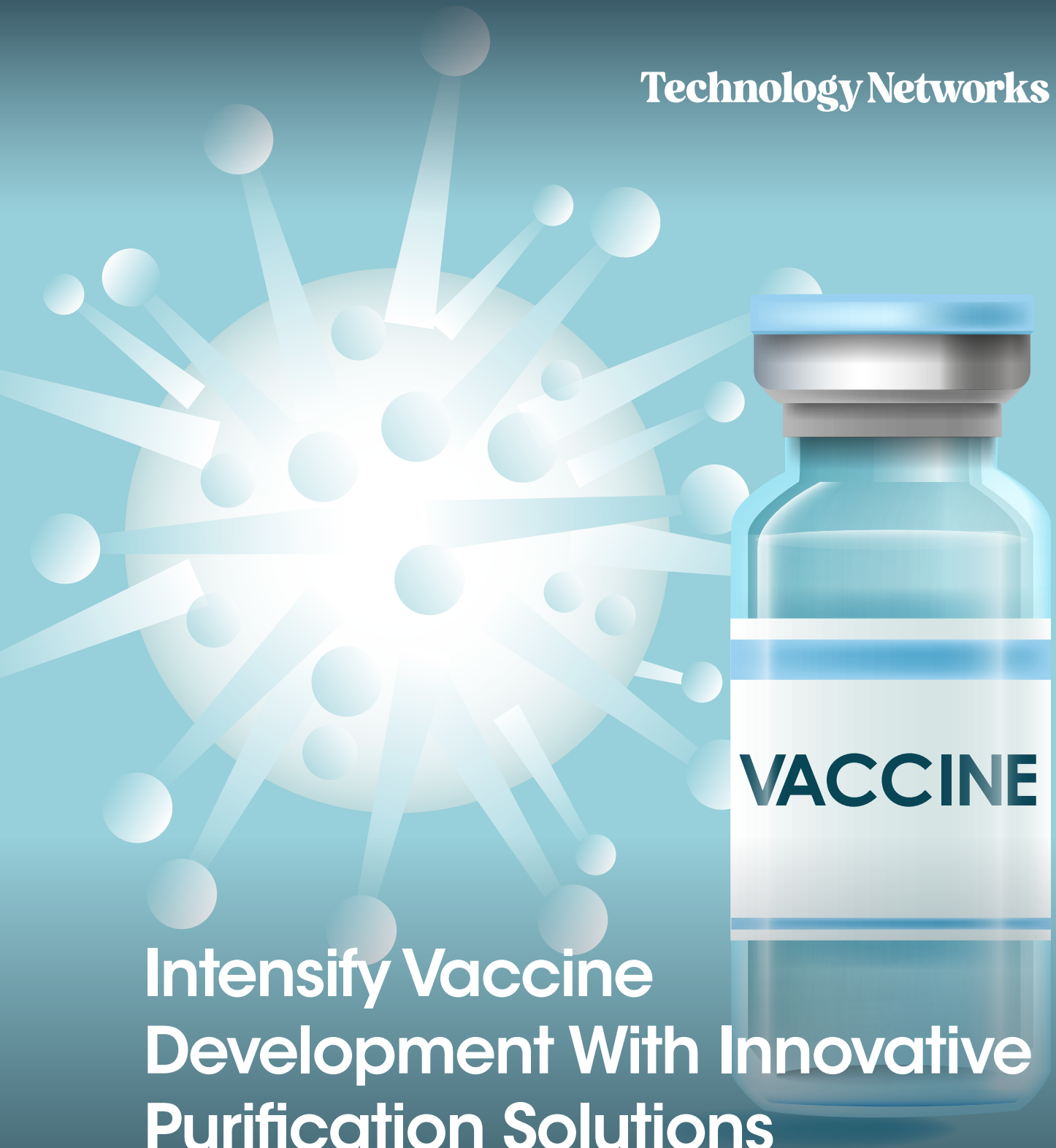
Technology Networks



Intensify Vaccine Development With Innovative Purification Solutions

A Closer Look at Vaccine Modalities

Accelerating Vaccine Development with Advanced Purification Tools

The mRNA Therapautics Boom

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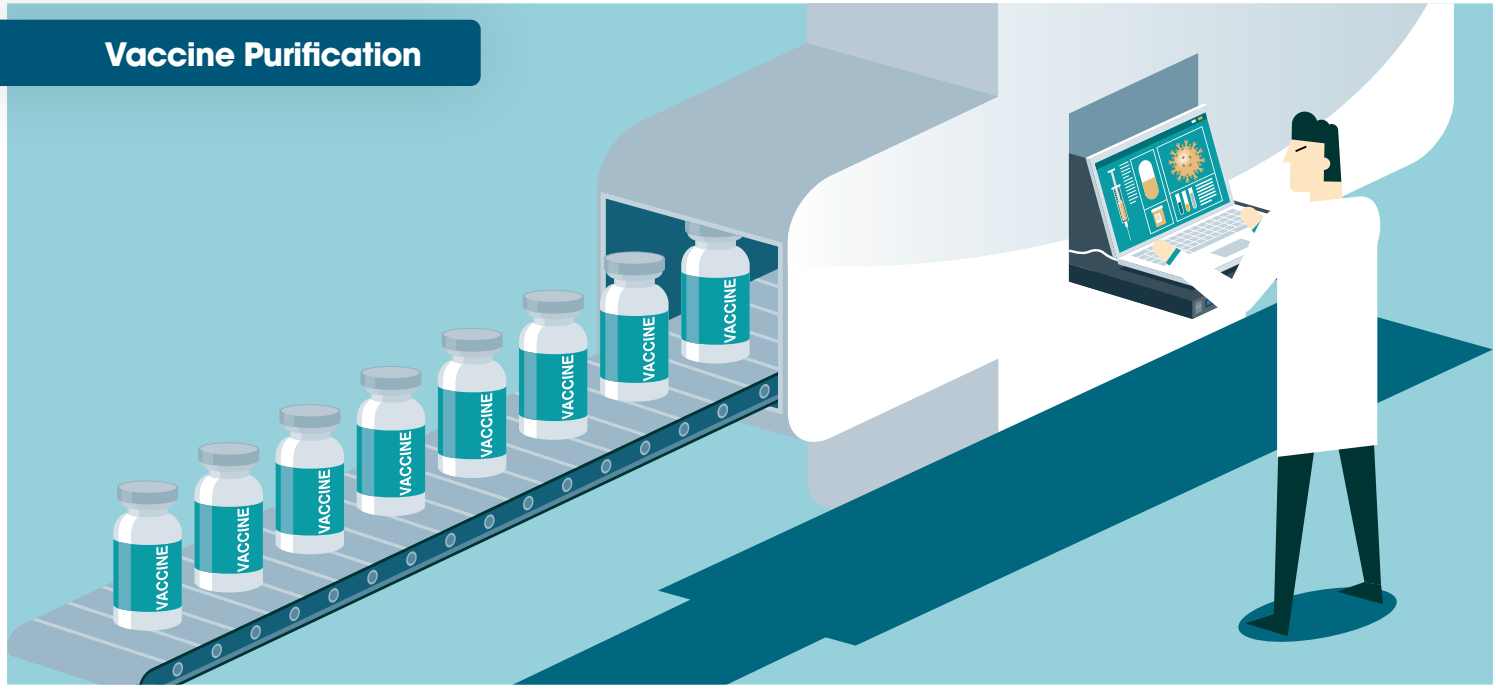
Foreword

Vaccine development has evolved tremendously in recent years thanks to the combined pressures of supply and demand, biological challenges, and innovations in the field. Although the development of new modalities, such as mRNA and DNA vaccines, offers greater safety and scalability than ever before, each type comes with its own challenges for manufacturing.

Innovative purification solutions are required to meet the global demand for all vaccine types while ensuring purity, safety and yield. This eBook will explore the landscape of vaccine production past, present and future, and highlight the various purification technologies that are available for therapeutic development.



Vaccine Production: Past, Present and Future

Introduction

The global vaccine development landscape has changed tremendously in recent years. Unprecedented levels of supply and demand, driven by the COVID-19 pandemic, have shifted development pipelines away from traditional methods.[1] Rapidly upscaled workflows and an increased range of modalities – including nucleic acids (DNA and mRNA), viral vectors, virus-like particles, protein sub-units, live-attenuated and inactivated virus vaccines – now offer solutions to a wide range of infectious and non-infectious disease challenges.[2,3]

Despite such advances, pathogen evolution remains a challenge for vaccine development. Whilst acquired resistance to established vaccines is considered a low-risk probability,[4] the emergence of new pathogen strains, to which humans have no natural immunity, continues to challenge public health. To ensure pandemic readiness and to secure vaccine equity, production efforts must focus on potent and effective solutions with minimal dosage requirements, and easy distribution through existing infrastructure networks.

Understanding past and present challenges in vaccine production can help researchers develop new and effective strategies to solve public health problems. This article explores the history of vaccine development, current trends and emerging future technology.

Vaccines and Immunity

Immunity refers to the body's ability to protect itself against harmful foreign agents, such as bacteria and viruses. The first line of defense is the innate immune response, a non-specific and quick response to any sort of pathogen. When needed a second defense mechanism, the adaptive immune response, is activated to neutralize the pathogen. As part of this response, specific lymphocytes produce antibodies which can bind to the pathogen to neutralize or signal for elimination from the body. The adaptive immune response comes with another benefit, immunological memory. The next time the same pathogen invades, the immune system can react faster and create specific antibodies to neutralize the pathogen. This response is the basis of vaccination.

Whilst several types of vaccines exist today, all work by stimulating the body's natural immune response. Vaccines promote the production of antibodies through the safe and controlled exposure to either a whole pathogen or pathogen fragments. This allows individuals to develop acquired immunity whilst minimizing the risk of disease. Immunotherapeutic vaccines containing tumor-associated antigens – proteins present on cancer cell surfaces – also work in the same way. They stimulate the production of specific antigens, allowing lymphocytes to recognize and destroy malignant cells. Whilst immunotherapy is established in the field of vaccinology, most current treatments remain in the clinical trial stages of development.[5]

Inactivated Vaccines
Pathogens are killed/inactivated with heat, chemicals, or reaction before being administered to the body to provoke an immune response.

Live-Attenuated Vaccines
A weakened form of the disease-causing germ is used to initiate a strong and long-term immune response within patients.

Subunit, Recombinant, Polysaccharide, and Conjugate Vaccines
A specific disease-causing piece of the germ like its protein, sugar, or capsid is separated out to create an immune response to that particular part.

Toxoid Vaccines
Disease-causing toxins made by the germ are administered. This provokes an immune response to the toxins and parts of the germ that lead to disease.

Nucleic Acid Vaccines
DNA, plasmid, and mRNA vaccines result in the endogenous generation of viral proteins that mimic antigens produced during natural viral infection.

The Early Years of Vaccinology

The origins of vaccinology are often traced back to the early 18th century – when the concept of inoculation was first discovered in Great Britain and North America. However, text records suggest that inoculation practices were being used widely across the African and Asian continents before their introduction to European communities.[6] Early methods of immunization involved the deliberate infection of individuals with a small dose of a pathogen under controlled conditions to reduce the risk of death by natural exposure. It was observed that controlled infection through cuts made in the skin – a process known as variolation – could reduce the number of deaths compared to infection by community transmission.[7]

The English physician Edward Jenner is credited as the father of vaccinology for his expansion upon inoculation methods. Jenner observed that infection with a lower-risk pathogen could offer protection against a similar, more deadly disease. His use of cowpox inoculant against the infection of smallpox in 1796 represented a historical step toward modern vaccine technology and set the precedence for the development of the first live attenuated vaccines.[8]

Live-attenuated vaccines contain a weakened version of a pathogen, capable of eliciting an immune response with a low probability of causing disease.[9] Inactivated vaccines – which use a killed version of the pathogen to elicit an immune response – can provide a safer option, although they provide shorter-lived immunity.[10] In 1885, the French microbiologist Louis Pasteur inoculated a nine-year-old boy, bitten by a rabid dog, using a neutralized form of the same disease, marking the first recorded usage of an inactivated rabies vaccine.[11] By the mid-20th century, inactivated vaccines had been developed for a range of diseases including typhoid and cholera.[12]

Mass Vaccination and Scaling-Up Production

During the early half of the 20th century, major advances in lab techniques enabled vaccine production to be scaled-up significantly to meet growing demand for mass immunization. Subsequently, a focus on vaccine refinement, efficacy and public appeal marked further developments in vaccinology.

In the 1930s, new methods of production focused on the growth of inactivated viral vaccines within serum-originated conditions.[13] Nutrient-rich serum provides an optimal environment for vaccine production and is still used to manufacture some flu vaccines today.[14] Yet, despite the success of serum-originated vaccines, extensive monitoring and safety requirements limit production flexibility. Over the last 70 years, developments in cell culture and recombinant technology have reduced the reliance on serum-originated workflows, increasing the productivity, yield and safety of vaccines.[15]

During the 1940s, the field moved away from the use of live-attenuated and inactivated vaccine modalities, towards the development of toxoid vaccines. These newer, partial pathogen modalities carried a lower risk and greater immunogenicity, allowing for the safe and effective rollout of vaccines against diphtheria, tetanus and pertussis (DTP) in children. With this development came the challenges of unprecedented supply and demand, as well as an increased need for public safety assurance.

Covalent (also known as recombinant) vaccine modalities offered greater public safety assurance, particularly in children and immunocompromised recipients, which possess a weaker immune system, and thus are not only at greater risk of infection but also of an adverse reaction to certain viral vaccines.[16] A reduced immune response in some immunocompromised patients also challenges the development of their adaptive immunity. First developed in the 1980s, covalent vaccines combined a weaker, lower-risk antigen with stronger carrier to elicit robust immunogenicity against bacteria such as *Haemophilus influenzae*.[17] Whilst the weaker antigen ensures that immune response is targeted, the stronger carrier ensures that immunogenicity is robust. Such was this development that it has enabled safe immunization of immunocompromised patients.[18]

Recombinant vaccine production involves the isolation and manipulation of genetic material to create or enhance desirable characteristics of target antigens, which are then expressed using host cells in industrial bioreactors.[19] Recombinant technology has formed the basis of numerous vaccines, including the vaccine against Human Papilloma Virus (HPV) which has been used to immunize millions of people worldwide.[20]

Vaccine Production on the World Stage

Viral vector vaccines use a modified version of a virus that is unable to cause disease to deliver genetic material of the target pathogen to the body. This genetic material encodes for specific antigens of the pathogen, which then elicit an immune response. Due to their predicted safety and high immunogenicity, viral vector vaccines have been used to prevent disease on the world stage.

During a Guinean outbreak of Ebola in 2016, almost 800 people were protected by recombinant viral vector vaccines.[21] The same Ebola vaccine was later licensed for large-scale vaccination approaches with success in reducing transmission. Whilst effective in the clinic, the mass manufacture of viral vector vaccines is not without its challenges.[22] During batch production, viable vectors can be lost due to the incomplete transfection of genetic material, reducing the economic viability of this modality.[23] Additionally, the risk of contamination from raw materials is a threat.[24] Single-use, scalable bioreactor systems have been developed to circumvent such issues.

Technological developments in next-generation sequencing also contributed to changes in global vaccine production, by providing real-time genomic surveillance on variant evolution and viral spread. For example, portable and rapid whole genome sequencing technologies were first used in 2014 to obtain pre-emptive, strategic vaccination against Ebola and feedback on vaccine efficacy and design against variants of concern.[25,26] This method was also deployed at a greater scale for mass immunization against SARS-CoV-2.[27]

The global need for vaccination during the SARS-CoV-2 pandemic re-set the focus to the speed of delivery and the minimization of dosage. Messenger ribonucleic acid (mRNA) vaccine production utilizes cell-free systems to synthesize large volumes of therapeutic grade product within bioreactor systems. The speed of production and low contamination risk makes mRNA vaccines a suitable option for present-day pandemic management and infectious disease control measures of the future. As a result, they now represent a substantial portion of the global therapeutic market segment and present a promising solution to many vaccine production challenges.[28] mRNA vaccines have been in development since the 1990s, however, their rapid degradation within the body meant that they made unsuccessful drug candidates until recent years.[29] Critical technological advancements in lipid nanoparticle delivery have only recently made mRNA vaccine technology a possibility. However, despite their initial promise, preparation, storage and cold-chain delivery limit their mass rollout globally.

The Future of Vaccine Technology

In light of COVID-19, the future of vaccine technology will likely focus on refining clinical efficacy, speed of delivery and increasing pandemic readiness. To circumvent the challenges of storage and transport, researchers are exploring the use of DNA vaccines that do not require cold chain protection. The first DNA vaccine, known as ZyCoV-D, was authorized for public use in India in September 2021 in response to COVID-19.[30] Additionally, DNA vaccines have shown success as immunotherapeutic agents in several tumor models.[31]

Alongside the emergence of new vaccine modalities, so too emerge new modes of delivery. Needle-free forms of delivery have been shown to stimulate more antigen-presenting cells than traditional intramuscular injections.[32] Such developments could see the introduction of 3D printing and sophisticated material engineering to vaccine filling and finishing pipelines.

Since the export of early inoculation practices from Africa and Asia into Europe, the field of vaccinology has helped to save countless lives worldwide. Vaccine development and production technology experienced a golden age of discovery during the 20th century, with genomics and epidemiology coming to prominence in recent years to further drive the field forward. The recovery and purification of vaccine products, also known as downstream processing, has enabled safe, effective and highly targeted delivery of prophylactic and curative healthcare to recipients across the globe.

Learn more about the latest vaccine purification technologies available

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A Closer Look at Vaccine Modalities

Various vaccine modalities exist today.[1] Deciding which modality is best suited to a particular application depends on several factors including pathogen type, immunogenicity, distribution and delivery.

Downstream processing (DSP) aims to achieve high purity and high yield. Yet, the increasing diversity of vaccine modalities requires specific purification solutions. This infographic will explore these modalities and potential purification solutions in more detail.

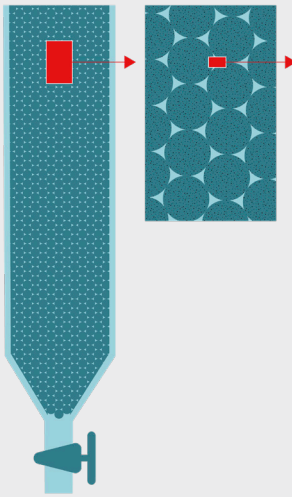
Vaccine downstream processing

Downstream processing of vaccines is aimed at removing impurities and contaminants from the culture media or feedstock. Purification processes must be designed in line with the properties of the specific vaccine modality in mind. Each modality encounters its own challenges in the downstream process. The development of a robust and efficient process is one of the major challenges in vaccine manufacturing.[2]

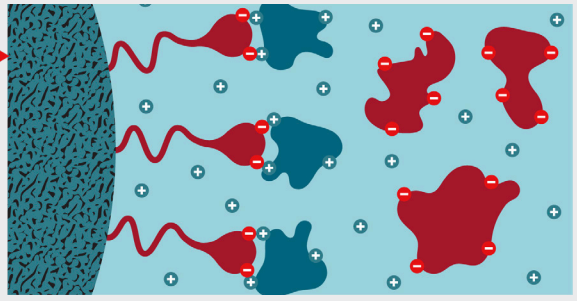
- Minimized complexity
- Reduced number of operations to increase the recovery and yield
- Scalable processes
- Good Manufacturing Practices (GMPs) compliance
- Flexibility and cost-efficiency

Purification methods

Chromatography is one of the most important steps in the downstream processing of biotherapeutics. It is the collective term for a set of separation techniques based on the interactions of the compound between a stationary phase (often a chromatography resin) and the mobile phase (the sample mixture). Ion exchange chromatography and affinity chromatography are two techniques often used in the DSP of various vaccines modalities.

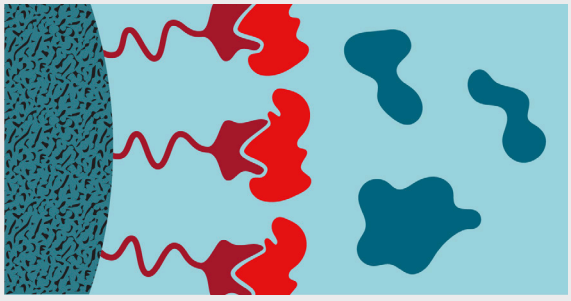


Ion exchange chromatography



Molecules are separated based on their surface charge. The process exploits the interaction between negatively or positively charged molecules in a sample and an oppositely charged chromatography matrix. Bound molecules are released from the matrix by increasing ionic strength or changing pH.

Affinity chromatography



Affinity chromatography is a separation method based on highly specific binding interactions between a target molecule and an immobilized affinity ligand on the stationary phase. Due to the high affinity of the target molecule, a higher yield and purity can be achieved in a single purification step.

Whole virus

Immune response triggered by	An attenuated (weakened) virus that can infect cells without causing disease[3] Or An inactivated virus, which is not infectious but can elicit an immune response
Pros	• Cost-effective[4] • Easy to produce[1] • Good immunogenicity
Cons	• Risk of infection[1]
Vaccine examples	Measles, mumps, rubella, hepatitis A
Purification challenges	Removal of pathogen contaminants from mammalian culture systems[8]
Purification solutions	**POROS™ IEX resins**

Viral vector

Immune response triggered by	A modified harmless virus carrying (a part of) the genetic material of the pathogen (following translation into protein by the host)
Pros	• Effective delivery[4] • Good immunogenicity[5]
Cons	• Pre-existing immunity toward the vector[1] • Challenging to purify[5] • Additional biosafety assessments are required[6]
Vaccine examples	COVID-19, ebola
Purification challenges	Removal of pathogen contaminants and disintegrated genetic materials from mammalian culture systems[9]
Purification solutions	**POROS™ CaptureSelect™ AAVX affinity matrix** **POROS™ CaptureSelect™ Adv5 affinity matrix**

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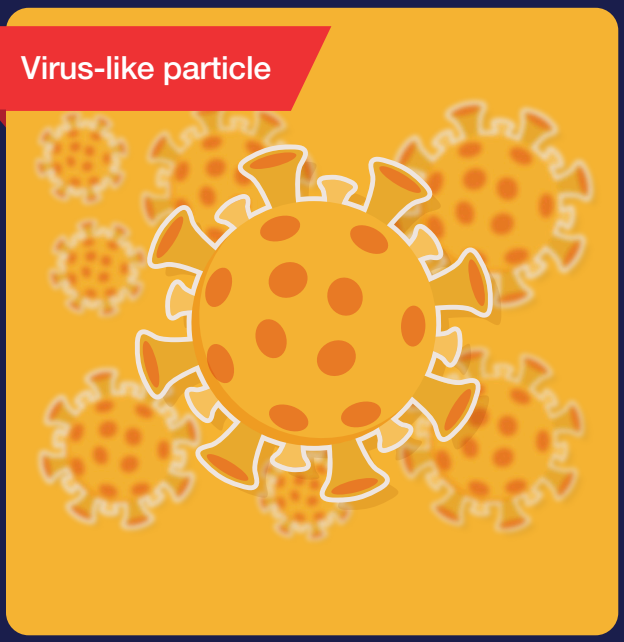
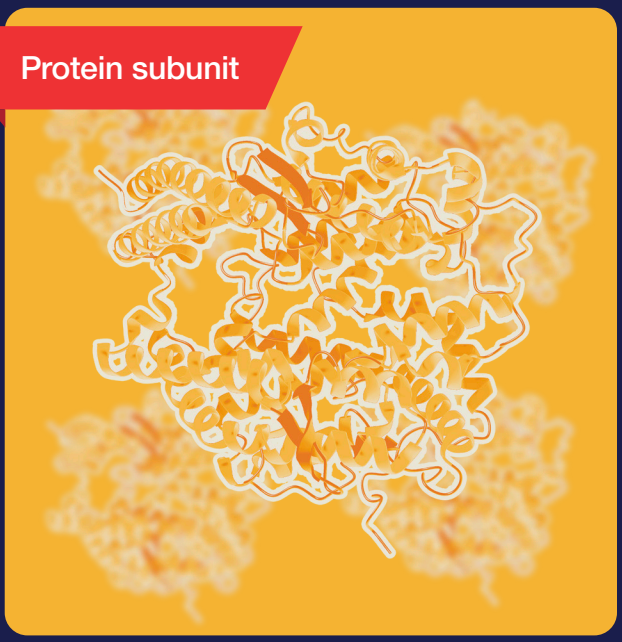
Virus-like particle



Immune response triggered by	The virus shell without genetic information (which cannot replicate)
Pros	• Safety • Quick to manufacture • Good immunogenicity
Cons	• Purification can be challenging and expensive[7]
Vaccine examples	HPV, hepatitis B & E
Purification challenges	Filtration of disintegrated protein fragments from particle suspensions[10]
Purification solutions	**Capture Select™ C-tagXL affinity matrix** **POROS™ HS resin** **POROS™ CaptureSelect™ BacuClear affinity matrix**

Protein subunit



Immune response triggered by	Fragments of protein from the pathogen
Pros	• Safety[5] • Stability[11]
Cons	• Expensive to produce[11] • Moderate immunity[5]
Vaccine examples	Whooping cough, hepatitis B
Purification challenges	Removal of pathogen contaminants from mammalian culture systems[8]
Purification solutions	**Capture Select™ C-tagXL affinity matrix**

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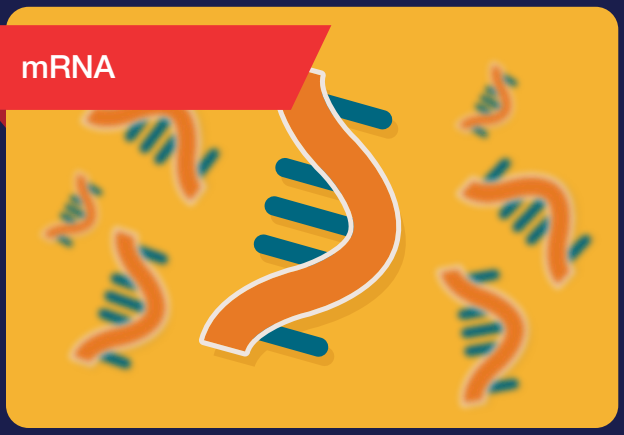
mRNA



Immune response triggered by	A mRNA sequence coding for a target antigen of the pathogen (following translation by the host)
Pros	• Safety[12] • Relatively easy to produce[5]
Cons	• Storage • Complex delivery[13] • Moderate immunogenicity[5]
Vaccine examples	COVID-19
Purification challenges	Denaturation of enzymes, NTPs and DNA template molecules in nucleic acid solutions[15]
Purification solutions	**POROS™ Oligo dT(25) affinity resin**

Plasmid DNA

Immune response triggered by	A DNA plasmid coding for a target antigen of the pathogen following translation by the host
Pros	• Relatively easy to produce • Good safety profile
Cons	• Vaccine delivery to the right cells has proven a key challenge[14] • Moderate immunogenicity
Vaccine examples	COVID-19
Purification challenges	Removal of other oligonucleotide molecules such as RNA and genomic DNA[16]
Purification solutions	**POROS™ AEX resins**

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Accelerating Vaccine Development and Large-Scale Manufacturing with Advanced Purification Tools

Recently, the technology used in vaccine development has seen significant improvements. As new vaccine modalities are on the rise, the purification process requires specific solutions tailored towards each unique molecule type.

Gain deeper insights and learn more about novel vaccine purification solutions. Accelerate early vaccine candidate screening and improve the downstream process of different vaccine modalities such as mRNA, recombinant proteins and virus-like particles.

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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*

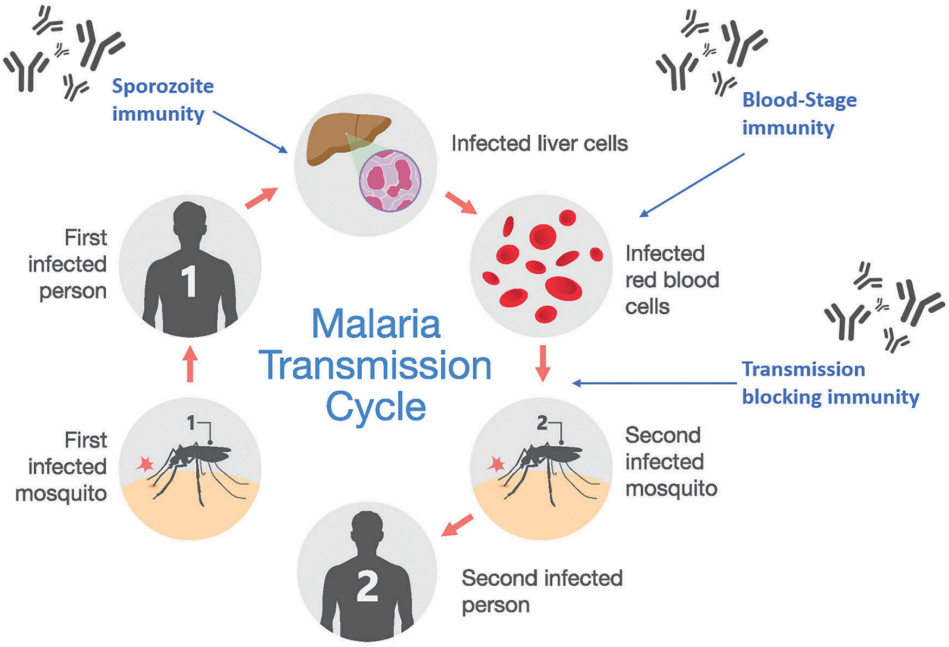
malaria transmission rates. This approval was the cumulation of over 30 years of research and development by GlaxoSmithKline, the nonprofit health organization Path, and other partners, including the Bill and Melinda Gates Foundation. “In field trials in Africa, RTS,S is approximately 30–50% effective,” 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?

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



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

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."

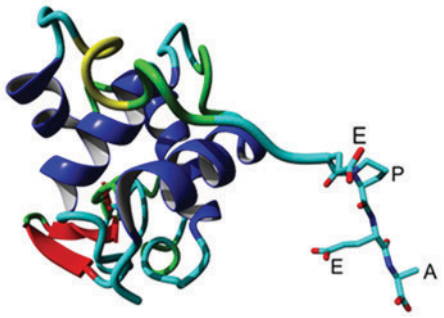
That lack of a biological target hinders researchers' ability to assess predictions of suitable vaccine designs in the lab. The Oxford team has concluded that clinical trials are the only appropriate way of evaluating hypotheses. "The overall strategy is to test as many different candidates as possible 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

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 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.

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 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.

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

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.

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 viruslike 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.

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The mRNA Therapeutics Boom

How to Simplify Workflows and Maximize the Efficiency of the mRNA Purification Process with POROS™ Oligo (dT)25 Affinity Resin.

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Scalable Purification of *In Vitro* Transcribed mRNA Accelerates mRNA-Based Therapy Development

mRNA is beautifully simple; it provides your body instructions. Analogous to computer code, mRNA programs the body to produce specific proteins giving this molecule utility in a myriad of therapeutic approaches, including vaccines against common and rare infectious diseases, oncology indications, and protein replacement treatments for genetic disorders. The wide diversity of mRNA-based therapeutic applications has led to increased interest in using synthetic mRNA.

In the early 90s, scientists demonstrated efficacy when using mRNA as a potential therapy.[1,2] But interest in antibodies, whose potential was more broadly accepted, took over in prominence. Today, mRNA

is back in focus with the general acknowledgment that these types of therapies do not target and manipulate genes and DNA.

To date, no mRNA-based therapy has been commercialized though some are in late-stage trials or approved for emergency use, such as vaccines targeting SARS-CoV-2. The vast majority of therapies in development apply to relatively small populations, thousands to hundreds of thousands of patients. Even mRNA-based cancer immunotherapies would serve a significantly smaller subset of patients than a global vaccination campaign.

Since preclinical and early clinical pipelines of most of these mRNA therapies only required a few liters of materials, traditional laboratory-scale approaches, such as precipitation, were leveraged for production.

"We are going to see mRNA therapies start to move quicker to the clinic," says Kelly Flook, PhD, Senior Product Manager, Purification Products, Thermo Fisher Scientific, "and a greater acceptance of protein replacement therapies and varied immunological approaches than are currently being evaluated."

Scaling-Up Options

The drive to rapidly develop a COVID vaccine put a focus on large-scale mRNA manufacturing. The limits of research-scale purification techniques were realized, and available purification methods became a bottleneck for commercialization.

To resolve this challenge, different options are under investigation. For example, scaling up reverse-phase chromatography is of interest. "It is scalable but not as efficient as an affinity approach in purifying the product and removing process impurities," says Flook. In addition, reverse-phase chromatography uses flammable solvents requiring the removal of detrimental post-purification impurities. Safety is a concern, as well as the necessity and expense of building a chemical manufacturing site to handle the solvents.

"Aqueous-based techniques, ion exchange, and affinity, are commonly used in research, and a similar solution is desirable for scale-up production of mRNA," says Flook. Process speed also plays an important role.

"A few years ago, as more companies began working on therapeutics in this space, we saw an increase in inquiries about large-scale mRNA purification," says Flook. "Most resins on the market were research-scale technology, such as our popular Dynabead option with a polyT on the surface. Initially, we provided custom resins until the momentum grew, and it made sense to develop a generic product. So we took the polyT technology and applied it to our bioprocessing POROS™ resins."

Producing mRNA

"RNA is made using a process called in vitro transcription (IVT). During the IVT process, DNA is converted to RNA," explains Venkata Indurthi, PhD, Vice President, Research and Development, Aldevron. "It is critical to get rid of all the impurities after the reaction is complete, including any residual raw materials, because they can trigger nonspecific immune responses."

Compared to DNA, RNA is fragile; harsh purification techniques are unsuitable. RNA also has secondary structures that can impact purification.

"There are multiple ways of purifying RNA, charge-based methods, precipitation-based methods, and others like hydrophobic interaction chromatography (HIC)," says Indurthi. "The specific advantage of the affinity oligo dT approach is that you can easily get rid of the impurities generated during IVT."

Affinity chromatography, a highly-scalable method, has earned its credits in the development of biologics, such as the use of Protein A for the purification of therapeutic antibodies and, more recently, anti-AAV resins in gene therapy workflows. An effective affinity purification step can help to simplify biomolecule downstream processing, reduce the number of purification steps, and lower the overall cost of goods in biotherapeutic manufacturing.

Thermo Fisher's new affinity-based mRNA chroma-

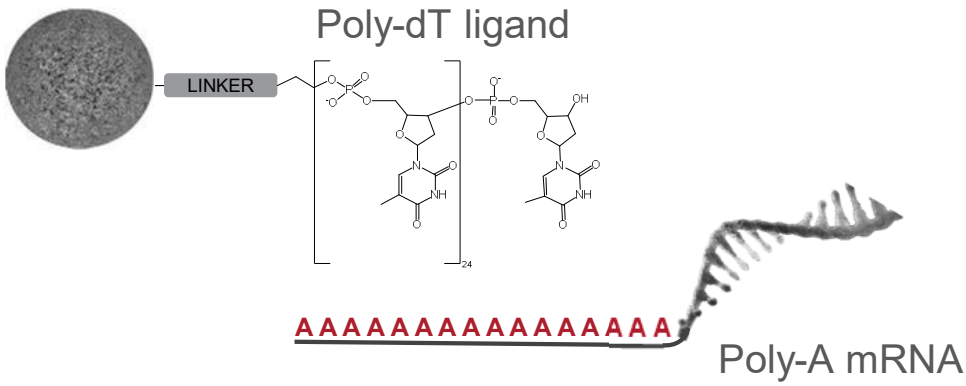

Fig. 1: POROS Oligo (dT)25 affinity resin consists of a polydeoxythymidine (dT-25) ligand attached to a 50µm rigid, porous bead through a proprietary linker. The poly-dT ligand allows binding with poly-A tailed mRNA molecules through AT base pairing.

tography resin, POROS Oligo (dT)25, was specifically developed for the scalable purification and isolation of mRNA from the IVT manufacturing processes.

"We worked closely with our customers to develop the resin, including AmpTec, a leading RNA CRO, in Europe," says Flook. "They were tasked to develop a scalable, efficient method for manufacturing mRNA that would allow them to take on large-scale vaccine manufacturing."

The Oligo dT Affinity Approach

POROS Oligo (dT)25 is based on POROS resin technology, a poly(styrene-divinylbenzene) base bead coated with a proprietary functional hydrophilic coating to reduce nonspecific binding. A dT-25–poly-deoxythymidine ligand is attached to the bead surface.

Since every mRNA has a polyA tail for molecular stability, the resin is a platform solution. "Across a range of mRNA sizes and constructs, you get equivalent recovery, purity, and yield," says Flook. "The size or sequence does not matter; the resin can be used to purify anything that has a polyA tail."

Use of the resin is straightforward; the polyT ligand on the bead binds to the polyA tail of the mRNA. In brief, hydrogen bonding occurs as salt neutralizes the backbone of the mRNA and the polyT, allowing flushing and removal of the non-bound IVT components. After the salt is removed, the hydrogen bonds break, and the polyA containing mRNA is eluted.

Typically, this affinity approach is used at the beginning of the purification scheme to remove process-related impurities, such as DNA templates, nucleotides, enzymes and buffer components, and other constituents such as mRNA without a polyA tail.

In some cases, a product-related impurity can result from IVT, such as double-stranded RNA (hairpin) or another undesirable species that has a polyA tail. "Then we suggest adding a second polishing step with ion exchange or HIC. The loop-back double-stranded effect can also be engineered out during the IVT process," says Flook. "Another optional way to use the resin is downstream as a final polishing step or for buffer exchange. You can elute in water and formulate directly from that."

Depending on the application, mRNA will vary in size and design of the backbone. "As a CDMO, Aldevron has been supplying RNA for a couple of years at all quality grades from RUO to GMP, and we are continuing to invest heavily in the space," says Indurthi. "RNA has a 5′ UTR and a 3′UTR and varying sequences.

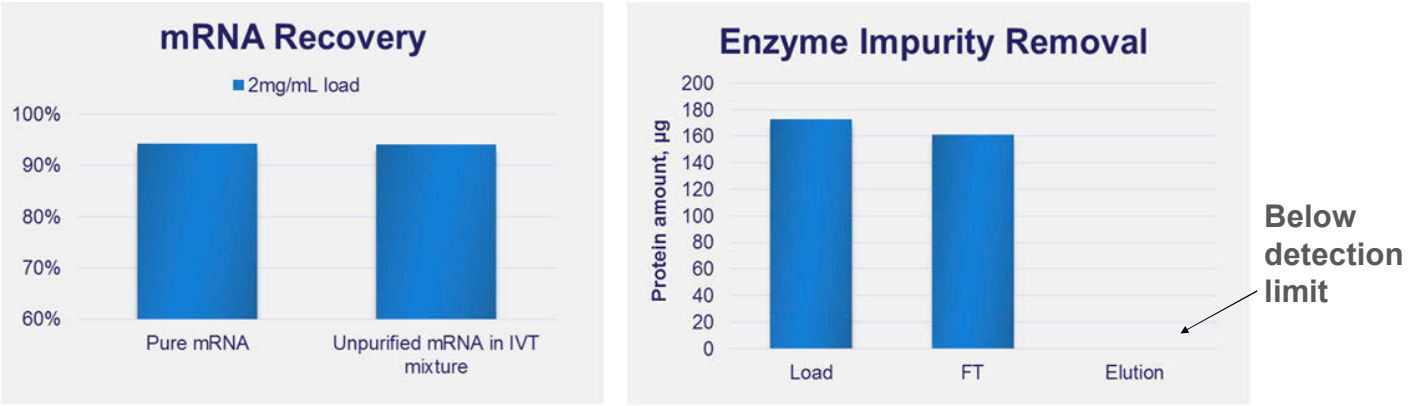

Fig. 2: High recovery and purity independent of sample type used. Recovery of mRNA from pure mRNA and unpurified IVT mixture, showed no differences (left). Amount of protein was determined in load, flow through (FT) and elution pools (right). No proteins were detected in the elution pool, indicating excellent impurity removal of IVT mixture products.

We do not control the design, and different designs can trigger complexity in the purification process."

The POROS Oligo (dT)25 resin, as a purification platform, can be cross applied to different constructs, emphasizes Flook. The resin addresses the current challenges involved with large-scale mRNA purification for potential clinical use by simplifying the downstream process, increasing purity and yield, and allowing for scale-up without the use of toxic chemicals.

Validating the POROS Oligo (dT)25 Resin

Standard mRNA contains between 1000-5000 nucleotides, which is the size the resin was designed to optimally operate in. Optimized conditions can maximize the binding capacity, even for larger RNAs, to achieve a more efficient purification process.

"From a binding perspective, we have seen customers achieving up to 5mg/ml of 4000 bp mRNA," says Flook. "This is significantly higher than what you would see with some of the research products."

A standardized experiment looked at the binding capacity of three different sizes of mRNA without optimizing conditions for each independently. Size did not impact recovery even with samples straight from an IVT mixture. Low nonspecific binding and the affinity approach only allow polyA species to bind. Recovery rates are greater than 90% and, in most cases, greater than 95%. Adjusting the column size according to need makes the process flexible and scalable.

From a purity perspective, evaluation of the proteinaceous load showed that primarily enzymes from the IVT mixture are seen in the flow-through but not detected within the elution peak. "If we analyze the fractions from the elution peak starting with about 17% product-related impurities, a reverse-phase spin column slightly reduces it to 13%," says Flook.

"All of the process-related components are removed with the POROS Oligo (dT)25 resin, and we see a significant reduction in product-related impurities," continues Flook. "This means our affinity resin does a much better job of removing non polyA species than reverse phase." All remaining product-related impurities are polyadenylated, as expected.

Overall, the POROS Oligo (dT)25 resin demonstrated efficient elution at different load concentrations and excellent recovery with high purity regardless of sample type.

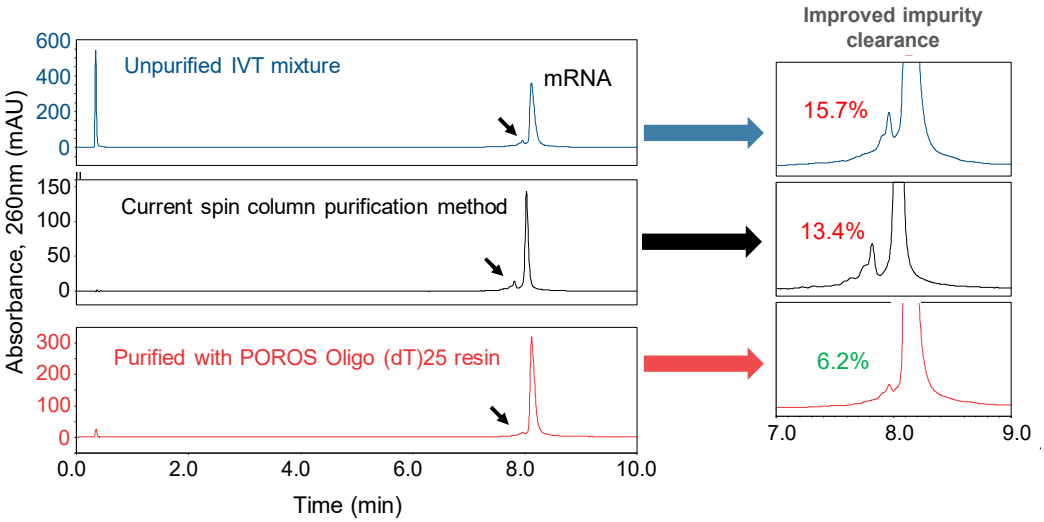

Fig. 3: Efficient removal of impurities compared to the spin column method. HPLC analysis of unpurified IVT mixture, spin column purified mRNA and mRNA purified with POROS Oligo (dT)25 resin.

"In reverse-phase, as the column is reused, the impurities start to follow the column, and that can change the column's effectiveness," says Flook. With POROS Oligo (dT)25 resin, the impurities are removed without first binding them to the column, rendering the number of impurities irrelevant. The resin is reusable and base stable for in-place sanitization.

"Each mRNA is designed for a specific application; the Cadillac version of purification is not always required. We have different purification strategies for different applications," says Indurthi. "As an example, if mRNA is being used for a vaccine, you do not need to get rid of certain impurities, but if you are making RNA for therapeutic use, you do because they will trigger an unwanted immune response."

In general, oligo dT purification can be used as a stand-alone purification. "We have seen pure RNA using this approach; it is a better way. The purer the end product, the less you need to get a response," says Indurthi. "Secondary structure, size, and other factors affect the recovery, not the resin itself."

"With all of the oligo dT resins we have tested, we observed that POROS Oligo (dT)25 has a very high binding capacity and provides the ability to purify larger mRNAs as compared to other products," adds Indurthi. "It works really well for our applications and will be added to our repertoire for mRNA production."

Looking to the Future

The COVID mRNA vaccines are expanding the already significant interest in the RNA space and taken mRNA manufacturing to a new level. Going forward, the biggest bottlenecks will be the DNA templates and the enzymes needed for synthesis. Luckily, the boom has also catapulted development activity in resins, nucleotides, and enzymes.

Hurdles still remain for mRNA therapeutics for different indications. One of the challenges is how to get the mRNA to the right cells, especially when targeting specific cancers. "We are going to see a lot of development around delivery systems," says Flook.

However, these challenges will not change the molecule's current trajectory. mRNA therapeutics are poised to become an important element in the healthcare landscape in the coming years. ■

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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 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.

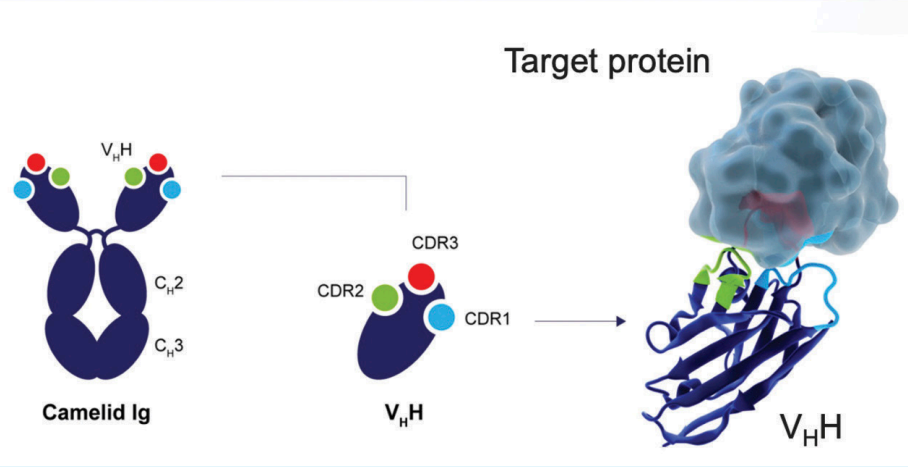
USING AFFINITY PURIFICATION FOR EFFECTIVE POLISHING

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.

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 sidebar) that specifically captures baculovirus particles and fragments, leaving virus like

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.

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.

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).

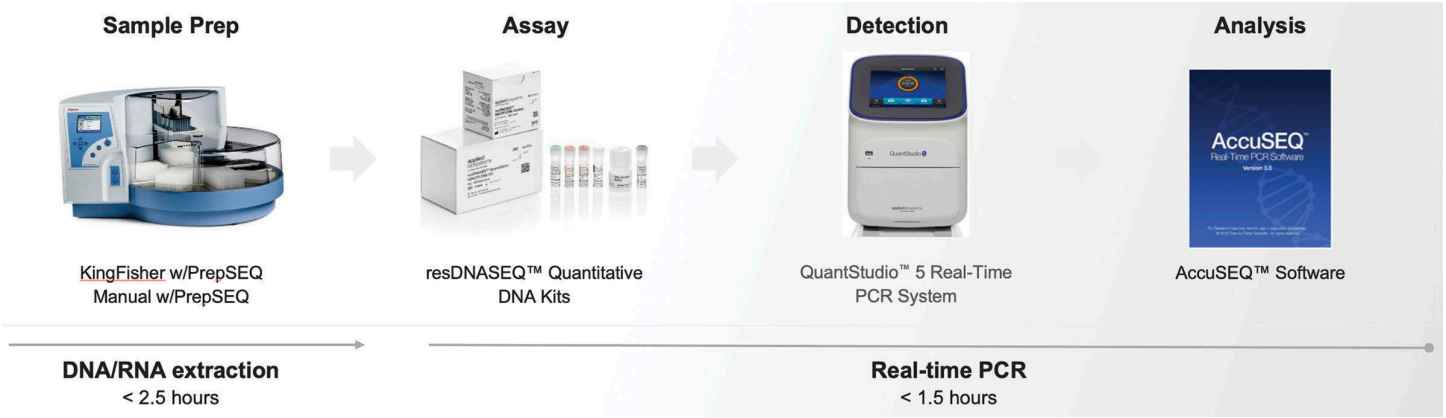


Figure 2: ResDNASEQ system integrates rapid sample preparation for nucleic acid extraction with a workflow for real-time quantitative polymerase chain reaction (PCR) analysis. Nucleic acid quantification can be used for rapid impurities and contaminant testing during regulatory quality control in biopharmaceutical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

Source: Thermo Fisher Scientific

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 a webinar here.

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